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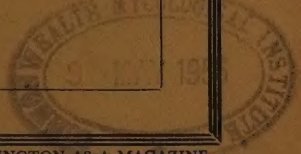

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(AGRICULTURAL RESEARCH SECTION)

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NOTICES

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NEW ZEALAND
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THE GALACTOPOETIC ROLE OF GROWTH HORMONE IN DAIRY CATTLE

By PETER J. BRUMBY and JOHN HANCOCK, Ruakura Animal Research Station, Department of Agriculture, Hamilton

(Received for publication, 21 September 1954)

Summary

1. Daily subcutaneous injections of growth hormone were administered to identical-twin cattle during (a) a 12-week therapy period over the peak phase of lactation, (b) a 4-week therapy period at the latter end of lactation.

2. In both trials a marked increase in milk and butterfat production resulted.

3. There was no change in either blood or milk composition due to growth hormone therapy *per se* in the treated animals.

4. Concomitant to the increased milk and fat yield there was an apparent increase in the efficiency of production.

5. The mechanism of the response, together with the physiological role that growth hormone may play in lactation, is discussed.

INTRODUCTION

In spite of considerable study of the problems of endocrine control of lactation, relatively little is known concerning the physiological role that the anterior pituitary plays in the maintenance of lactation. For some years it has been known that crude extracts of the anterior pituitary are capable of stimulating established lactation[1], while quite recently Cotes *et al.* have brought forward evidence implicating growth hormone as the galactopoetic factor in such extracts[2]. Since then brief reports have appeared relating the administration of growth hormone to the stimulation of milk yield[3,4].

In view of the physiological implications that are involved in a galactopoetic response to an anterior lobe factor, it was decided to investigate this apparent galactopoetic activity of growth hormone rather more critically than has been possible to date.

Two main trials have been carried out. The first involved a 12-week therapy period throughout the peak phase of lactation, the animals being fed grain plus good quality pasture *ad lib.* The second involved a 4-week therapy period during the declining phase of lactation, the animals being fed on good quality pasture only. A considerable quantity of a growth hormone preparation (Batch No. R.527094B) was kindly supplied by Armour Laboratories, thus making this project feasible.

TRIAL 1

EXPERIMENTAL

Dose Rate

A series of preliminary pilot experiments carried out with lactating identical-twin cattle in the latter part of lactation had demonstrated a marked galactopoetic response when subcutaneous doses of the order of 50 mg. of growth hormone were administered daily. Doses of a lower order appeared to bring about a lesser response, whereas 100 mg. doses appeared little superior to doses of 50 mg. Thus it was decided to use 50 mg. as the standard daily dose. The growth-hormone preparation employed was known to contain thyrotropic hormone in a concentration of 30 units T.S.H. (U.S.P. standard) to each 100 mg. growth hormone. Consequently, a third group of twins was injected throughout the experimental period with 15 T.S.H. units per day. After eight weeks of thyrotropin therapy, supplies of this preparation were exhausted. For the remaining four weeks of the therapy period L-thyroxin was used in its place; the dose rate used was 0.5 mg. per 100 lb. liveweight.

Animals

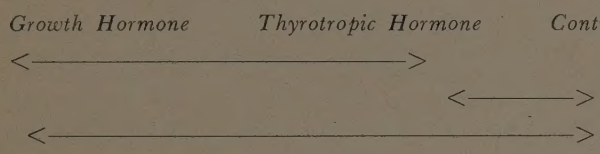
Nine sets of mature identical-twin cattle of predominantly Jersey origin were used in this trial. The animals selected were expected to reach their normal lactational peak production during the 12-week course of daily growth-hormone therapy. Table 1 records the date of birth, date of calving, number of days from calving to first injection, breed, and treatment of each of the eighteen animals involved.

TABLE 1. DETAILS OF EXPERIMENTAL ANIMALS

Cow No.	Age (years)	Date Calving	No. Days from Calving to First Injection	Breed	Treatment
37	8	10/8/53	52	Ayrshire	Growth hormone
38		1/8/53	60	„	Control
43	8	27/6/53	82	Shorthorn × Jersey	Growth hormone
44		27/6/53	81	„ „	Control
77	7	26/7/53	53	Jersey	Control
78		1/8/53	47	„	Thyrotropic hormone
167	5	24/7/53	55	Shorthorn × Ayrshire	Thyrotropic hormone
168		8/8/53	40	„ „	Growth hormone
185	5	24/7/53	54	Jersey	Growth hormone
186		5/8/53	43	„	Thyrotropic hormone
221	4	13/7/53	79	Jersey	Control
222		11/7/53	80	„	Thyrotropic hormone
255	3	4/8/53	56	Shorthorn	Control
256		23/7/53	46	„	Thyrotropic hormone
267	3	27/7/53	52	Jersey	Growth hormone
268		22/7/53	58	„	Control
287	3	2/9/53	22	Jersey × Ayrshire	Thyrotropic hormone
288		22/8/53	40	„ „	Growth hormone

Design of Experiment

As the experiment required the use of three groups of cattle, the nine sets of identical twins available were divided to form three replications of a balanced, incomplete block. Thus, for each replication three twin sets were divided as follows:



Feeding

All cows were fed *ad lib.* on good pasture as well as being provided with meal at the rate of 1 lb. per 5 lb. of milk yielded. Meal feeding continued throughout the therapy period and for six weeks thereafter. The meal fed consisted of a mixture of four parts of crushed oats to one part of bran.

Responses Measured

Milk and fat yield. Milking was carried out under standardized milking conditions. Milk yields were recorded twice daily and a composite sample of the morning and evening milk tested daily for fat content. Once weekly, a one-day composite sample was analysed for solids not fat, (S.N.F.), protein, and lactose. These latter analyses were carried out by Dawn R. Perrin, to whom the authors are greatly indebted.

Heart rate. Throughout the treatment period and for four weeks thereafter heart rate was recorded twice weekly by direct auscultation of the heart during four 30-second periods.

Body weights. These were measured weekly.

Blood composition. Twice-weekly estimates were made of blood glucose in all animals. In two of the three replication groups ketone bodies and non-protein nitrogen (N.P.N.) were also estimated. The blood analysed was a composite a.m./p.m. sample taken from the jugular vein. These analyses were carried out by R. R. White, to whom the authors express their thanks.

Intake. During the latter part of the therapy period an attempt was made to estimate the intake of grass over two seven-day periods. The calculation of grass intake was based on the following scheme:

(i) Output of faeces organic matter by each cow estimated from Cr_2O_3 in grab samples[5]. All cows were dosed with 10 gm. Cr_2O_3 twice daily commencing $4\frac{1}{2}$ days before the first sample.

(ii) Digestibility of grass consumed by meal-fed cows estimated from nitrogen in faeces from 6 cows receiving no meal but grazing with the meal-fed cows[6].

(iii) Total intake of meal organic matter by each cow (measured).

(iv) Digestibility of meal (estimated 80%).

From (iii) and (iv) the contribution of meal faeces to total faeces excreted by each cow receiving meal could be deduced. Using the intake factor for grass derived from (ii) and total faeces from (i), an estimate of the intake of digestible organic matter (D.O.M.) from grass could be calculated. For these estimations the authors are greatly indebted to Messrs. M. R. Coup and R. L. Lancaster.

Grazing observations. During the period in which intake measurements were being conducted, grazing observations were carried out. These comprised grazing, cudding, idling, and resting times, during a total of four 24-hour periods within the fortnight for which grass intake results are reported.

RESULTS

Milk Yield

The commencement of daily growth-hormone therapy resulted in a rapid and marked increase in daily milk yield, this increase continuing for approximately six weeks (Fig. 1). Thereafter the milk yield of all three groups tended to decline in a parallel manner. With the cessation of the therapy treatment, the milk yield of the growth-hormone group declined, reaching the level of the control animals within three weeks and remaining at about this level from then on. There was no tendency for the yield of the growth-hormone animals to drop below the level of the controls once therapy ceased. The commencement of thyrotropin therapy was followed by a slight increase in milk yield, this increase being apparently unaffected by the changeover from thyrotropin to L-thyroxin therapy at the eighth week of the injection period. In contrast to the growth-hormone group, the production of the thyroxin group dropped below that of the controls once thyroxin therapy ceased.

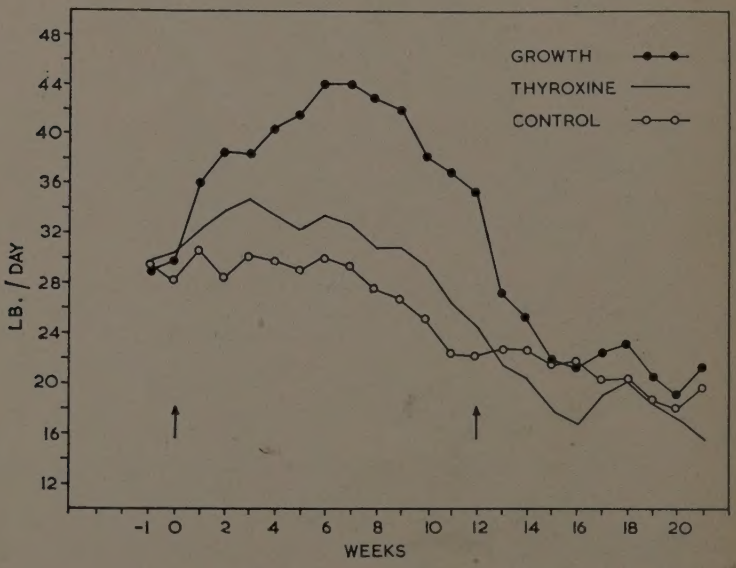


Fig. 1.—Average daily milk yield on a weekly basis.

The total yield of milk recorded for each animal during the injection period is tabulated in Table 2. Table 3 records the total production of each animal for the 8 weeks following the cessation of injections.

TABLE 2. TOTAL MILK YIELD FOR 12-WEEK INJECTION PERIOD (LB. MILK)

Set	Growth Hormone	Thyrotropic Hormone	Control
37–38	4211		3793
43–44	2370		1219
267–268	4016		2310
167–168	3497	2227	
185-186	4026	3756	
287–288	2029	1813	
77-78		2827	2352
221-222		186	235
255–256		2385	1830
	20149	13194	11739

TABLE 3. TOTAL MILK YIELD DURING 8-WEEK PERIOD FOLLOWING CESSATION OF INJECTIONS (LB. MILK)

Set	Growth Hormone	Thyrotropic Hormone	Control
37–38	1828		2070
43–44	393		336
267–268	1529		1221
167–168	1226	764	
185–186	1649	1669	
287–288	248	220	
77–78		1133	1262
221–222		—	—
255–256		877	815
	6873	4663	5704

An analysis of variance carried out on these data indicated that the apparent increase in the milk yield of the thyrotropin-thyroxin group during the therapy phase was non-significant at $P < 0.05$, but with such a limited number of degrees of freedom available in the analysis this result was to be expected. The increase in milk yield of the growth-hormone group proved to be highly significant in spite of the limited number of animals involved. During the subsequent 8-week period

the apparent difference in production between the three groups was non-significant. Analyses of variance carried out on these data, but excluding the very low-producing set T.221, T.222, gave substantially the same result. Unfortunately, estimates of genotype–treatment interaction cannot be obtained from split twin experiments that are not time replicated. However, it is apparent from Table 2 that there was appreciable variation between sets in their response to therapy treatment.

Butterfat Yield

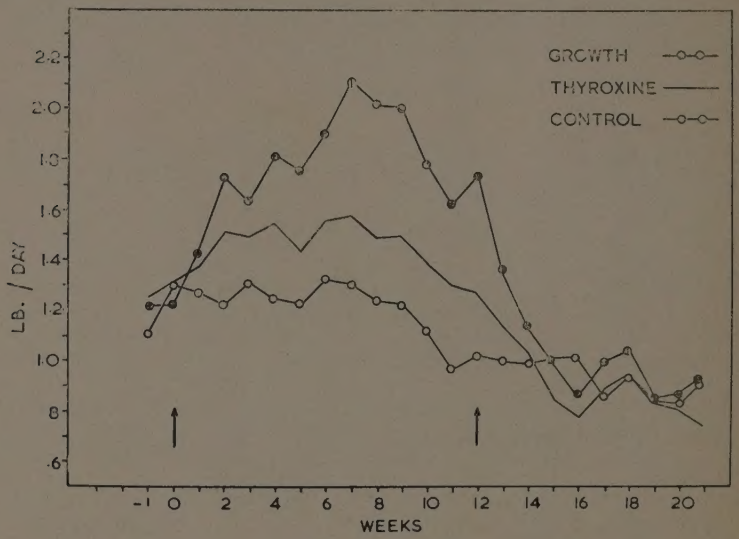


Fig. 2.—Average daily butterfat yield on a weekly basis.

TABLE 4. TOTAL BUTTERFAT YIELD FOR THE 12-WEEK INJECTION PERIOD (LB. FAT)

Set	Growth Hormone	Thyrotropic Hormone	Control
37–38	178.3		151.7
43–44	114.7		100.9
267–268	171.9		100.9
167–168	168.6	104.9	
185–186	174.9	154.7	
287–288	95.5	89.7	
77–78		145.0	115.1
221–222		9.6	12.7
255–256		113.1	80.6
	903.9	617.0	561.9

The response, in terms of butterfat yield, of the three groups of cattle closely simulated that for milk yield (Fig. 2). Total butterfat yields of all cattle for the injection period and post-injection phase may be found in Tables 4 and 5 respectively.

TABLE 5. TOTAL BUTTERFAT YIELD DURING THE 8-WEEK PERIOD FOLLOWING THE CESSATION OF INJECTIONS (LB. FAT)

Set	Growth Hormone	Thyrotropic Hormone	Control
37–38	75.7		88.5
43–44	17.6		14.2
267–268	68.9		52.5
167–168	55.2	34.2	
185–186	80.5	80.4	
287–288	14.4	11.2	
77–78		59.9	65.0
221–222		—	—
255–256		42.5	40.5
	312.3	228.2	260.7

The results of analyses of variance on these data are substantially the same as for those performed on the milk-yield data. In the case of butterfat yield, however, it is interesting to note that the variance ratio value of the thyroxin–control comparison is significant at the level $P < 0.1$. As was the case with the milk-yield data, the exclusion of the low-producing sets T.221, T.222, did not alter the results of these analyses.

Butterfat Test

A comparison of the average weekly fat test appeared to indicate that the thyrotropic-hormone therapy resulted in an increased fat content of the milk (Fig. 3). An analysis of variance carried out on these data, however, failed to reveal any significant difference in fat test between the three groups.

Protein, Lactose, and S.N.F.

There were no differences between the three groups. Mean percentage yields of each animal for each component are tabulated in Table 6.

Heart Rate

The average heart rate of the three groups, plotted as a moving average of five observations, is recorded in Fig. 4. Both the growth-hormone and thyrotropic-hormone groups exhibited a significant increase in heart rate compared with the control group, there being no significant difference between the two treatment groups, however. Once therapy ceased, there was a rapid drop in heart rate, the thyroxin group apparently diminishing at a slightly greater rate and to a somewhat lower

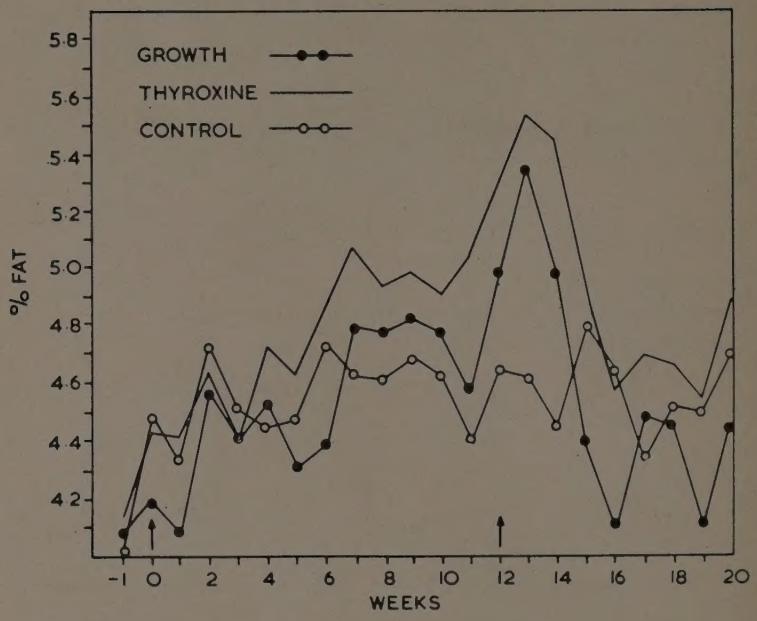


Fig. 3.—Weekly butterfat percentage.

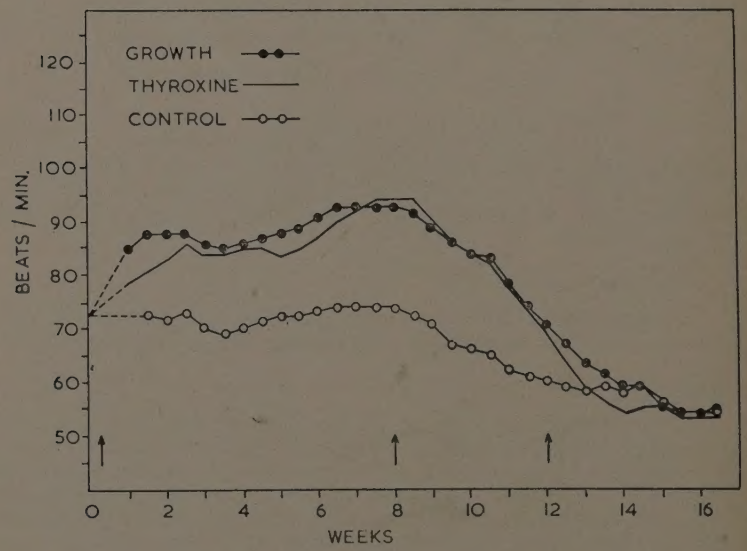


Fig. 4.—Smoothed heart rates per minute; average for groups.

TABLE 6. MILK COMPOSITION DATA: SUMMARY OF WEEKLY ESTIMATIONS

Twin No.	Treatment	12-Week Therapy Period			8-Week Post-therapy		
		Protein % (N.x 6.38)	Lactose %	S.N.F. %	Protein % (N.x 6.38)	Lactose %	S.N.F. %
37	G.	3.49	4.24	8.50	3.58	4.27	8.61
38	C.	3.54	4.52	8.82	3.49	4.54	8.76
43	G.	3.74	4.74	9.34	4.49	3.52	8.78
44	C.	3.96	4.38	9.13	3.78	3.63	8.22
267	G.	3.59	4.90	9.27	3.63	4.88	9.25
268	C.	3.67	4.77	9.03	3.49	4.68	8.92
78	Th.	3.79	5.01	9.52	3.85	4.89	9.47
77	C.	3.84	4.73	9.31	3.84	4.74	9.36
222	Th.	4.18	2.86	7.87	—	—	—
221	C.	4.39	2.99	8.22	—	—	—
256	Th.	3.69	4.71	9.14	3.76	4.51	9.00
255	C.	3.95	5.02	9.79	3.72	4.44	8.90
168	G.	3.35	4.72	8.83	3.60	4.71	9.03
167	Th.	3.46	4.68	8.88	3.42	4.54	8.71
185	G.	3.76	4.86	9.22	3.80	4.71	9.27
186	Th.	3.64	5.05	9.43	3.64	4.86	9.27
288	G.	3.67	4.31	8.74	3.75	3.12	7.62
287	Th.	3.77	4.50	9.02	3.80	2.71	7.32

level. This situation resulted, no doubt, as a consequence of the substitution of L-thyroxin for thyrotropic hormone at the eighth week of therapy. Within a matter of three weeks after the cessation of injections all three groups were again identical in this function. It should be noted that the apparent rapid drop in the two treatment groups just before the end of the treatment period is a result of using a moving average. In reality the three curves parallel each other reasonably well up to the point where therapy ceased. The point at which L-thyroxin was substituted for thyrotropic hormone is indicated in Fig. 4. Some slight elevation in heart rate may have resulted from this changeover.

Body Weight

A plot of the smoothed five-weekly moving-average body weight of each group is presented in Fig. 5. It is apparent that there was no change in the relative body weights of the control and growth-hormone groups, whereas the thyroxin group failed to make the same body-weight gain as the other two groups. This difference in body weight proved to be statistically significant. Once therapy ceased, the thyroxin group

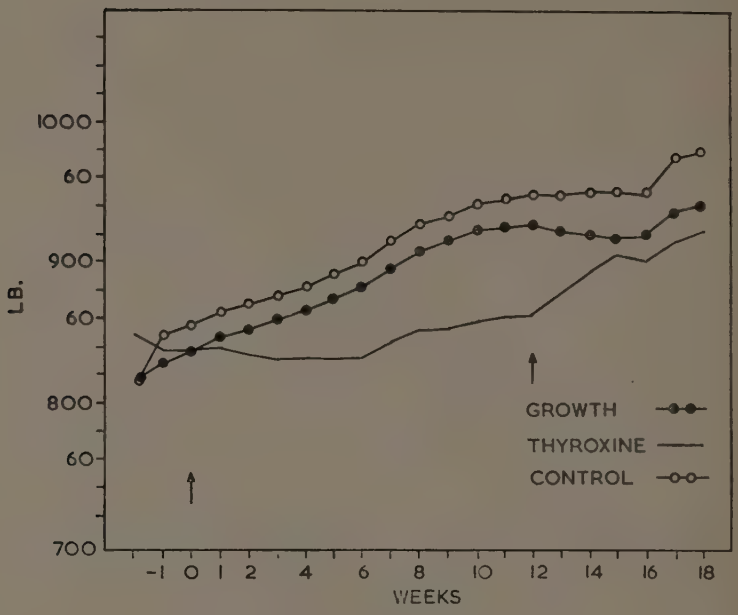


Fig. 5.—Smoothed body-weight changes.

gained weight rapidly in comparison with the other two groups. Unfortunately, it did not prove possible to obtain accurate data concerning changes in body composition.

Blood Composition

Blood glucose. The blood-sugar level of both treated groups was raised appreciably as a result of the daily injections. This increase was of the order of 25% (Table 7) and proved to be statistically significant. As there was no difference in the magnitude of response between the two therapy groups, the increase in blood sugar in the growth-hormone group could be attributed to the thyrotropic-hormone contamination of the growth-hormone preparation.

Total acetone bodies and N.P.N. Though the results are not sufficient in number to present a clear-cut picture, it would appear that in both hormone-therapy groups the concentration of total acetone bodies increased. It would seem likely that the thyrotropic activity in the growth-hormone preparation may account for its activity in this respect. In contrast to the blood acetone bodies, there appeared to be no marked alteration in the level of blood N.P.N., save in the case of the twin No. 43. The kidneys of this animal later proved to be pathologically abnormal due to a leptospiral infection, thus it is probably wise to discount the result presented by this animal. It is of interest to note

TABLE 7. BLOOD COMPOSITION DATA: SUMMARY OF WEEKLY ESTIMATIONS

Twin No.	Treatment	Blood Glucose mg. %	N.P.N. mg. %	Total Acetone Bodies mg. %
37	G.	53.0	—	—
38	C.	46.6	—	—
43	G.	69.4	3.60	54.7
44	C.	46.1	2.50	34.8
267	G.	50.8	3.53	33.7
268	C.	46.8	2.88	33.9
78	Th.	52.3	4.10	31.2
77	C.	45.2	3.54	36.3
222	Th.	58.6	—	—
221	C.	45.3	—	—
256	Th.	56.8	2.70	36.8
255	C.	42.8	2.51	36.0
168	G.	57.8	3.56	30.3
167	Th.	48.8	2.99	33.8
185	G.	48.7	2.79	32.0
186	Th.	57.9	3.11	30.9
288	G.	64.1	—	—
287	Th.	67.4	—	—

that in further trials at Ruakura the influence of growth hormone *per se* upon blood sugar and N.P.N. was likewise negligible when administered daily to young fully fed calves. This unaltered level of blood sugar and N.P.N. was also unaccompanied by any concomitant increase in growth rate of the calves.

Intake

Table 8 records the estimate of D.O.M. intake for each cow during the last fortnight of the trial. In the same table is listed the production of each animal in terms of fat-corrected milk (F.C.M.), as well as the animal's liveweight and maintenance requirement. This latter estimate is based on the figures derived at Ruakura by Wallace for the maintenance requirement of dairy cattle under free-grazing conditions. Net efficiency estimates of milk production for each animal have thus been derived. From these figures it appears that an appreciable difference in the efficiency of milk production existed between the growth-hormone and control groups.

TABLE 8. YIELD OF MILK IN RELATION TO FEED INTAKE (TRIAL 1)

Twin No.	Yield (lb. F.C.M.)	Intake (lb. D.O.M.)	Liveweight (lb.)	Maintenance Requirement (lb. D.O.M.)	F.C.M. Net Intake	Treatment
37	668.6	515.0	900	147	1.82	G.
38	519.6	515.2	870	143	1.40	C.
43	397.5	350.2	930	154	2.03	G.
44	112.8	330.7	1110	155	0.64	C.
267	670.1	433.6	860	146	2.33	G.
268	365.8	381.3	860	146	1.55	C.
78	523.9	386.3	820	141	2.13	Th.
77	388.4	380.4	920	151	1.70	C.
222	—	241.8	970	157	—	Th.
221	—	245.1	960	156	—	C.
256	412.1	324.9	820	141	2.24	Th.
255	272.2	317.2	970	157	1.70	C.
168	584.9	484.9	1040	165	1.83	G.
167	318.2	361.0	940	154	1.54	Th.
185	694.3	495.2	950	155	2.04	G.
186	549.3	528.9	860	146	1.43	Th.
288	287.5	325.6	880	148	1.62	G.
287	237.5	348.3	800	137	1.13	Th.

G.—Growth Hormone; Th.—Thyrotropic Hormone; C.—Control;
D.O.M.—Digestible Organic Matter; F.C.M.—Fat-corrected Milk

Grazing Observations

The daily average duration time of each of the four activities: grazing, cudding, idling, and resting, recorded throughout the four 24-hour periods in which the cattle were watched, is tabulated in Table 9. In the same table is listed the actual portion of the total D.O.M. intake of each animal derived from grass alone during the fortnight in which grazing observations were made. As pointed out by Hancock[7] the idiosyncrasies of individual animals in regard to their grazing and cudding rates are such as to prevent any significant correlation between these two characteristics and intake when compared under normal free-grazing conditions. With identical-twin cattle, however, group comparisons using limited numbers became possible. Thus in the grazing data presented it may be noted that whilst there is no close relationship between either grazing and cudding time, and D.O.M. intake for individual animals, a comparison on a group basis indicates a parallel pattern between the intake estimates and grazing behaviour. This relationship affords a measure of reliability for the intake data presented.

TABLE 9. SUMMARY OF GRAZING OBSERVATION DATA

Twin Set	Growth Hormone					Thyrotropic Hormone					Control				
	Grazing	Cudding	Idling	Resting	D.O.M. Intake	Grazing	Cudding	Idling	Resting	D.O.M. Intake	Grazing	Cudding	Idling	Resting	D.O.M. Intake
37–38	519	535	237	148	191						491	525	283	142	195
43–44	469	511	310	150	134						455	509	291	185	138
77–78						441	566	241	193	148	434	565	241	200	149
167–168	423	534	279	204	185	407	512	312	209	140					
185–186	497	496	260	188	189	540	536	239	125	205					
221–222						394	525	252	269	107	446	471	249	274	109
255–256						503	500	256	177	122	499	475	265	201	122
267–268	521	542	201	176	158						429	511	256	244	150
287–288	425	564	197	255	126	484	507	242	208	138					
Totals	2854	3182	1484	1121	983	2774	3146	1542	1181	860	2754	3056	1585	1246	863

TRIAL 2

The results of the first trial presented several rather anomalous situations. The very large milk-yield response of the treated animals over the peak phase of lactation was indeed surprising, likewise the increase in the net efficiency of production. A possible complicating feature of these results, however, was the fact that all animals were fed grain according to the level of production, as well as pasture *ad lib.* To check this feature of the results a further experiment was carried out using twins approaching the end of a lactation period and fed on grass alone. This further trial also provided an opportunity of checking the influence of growth hormone on blood-sugar levels and efficiency estimates.

EXPERIMENTAL

Four sets of mature identical-twin cattle were used, these animals having been previously involved in Trial 1. At the time of the first injection these animals had been in milk for approximately 265 days. The dose rate of growth hormone was the same as that employed in Trial 1, namely 50 mg. per cow per day. A thyrotropic hormone group comparison was not made owing to limitations of the number of twins available.

Milk yields were measured daily. Three times a week an a.m./p.m. composite milk sample was tested for fat content. Commencing 5 days after the first injection, the intake of digestible organic matter, derived entirely from pasture, was measured weekly throughout the three subsequent weeks. Intake estimates were based on the chromium oxide marker, nitrogen digestibility technique[5] [6]. For these estimates the authors are indebted to M. R. Coup.

The cows were bled twice weekly and blood glucose estimations carried out by R. R. White, using the Somogyi method.

RESULTS

Milk and Fat Yield

As in Trial 1 the commencement of daily growth hormone therapy resulted in a rapid and marked increase in daily milk and fat yield (Tables 10 to 15). Once peak yields had been attained in the growth-hormone group, these animals declined in production in a manner parallel to the control group. The cessation of daily therapy resulted in a marked diminution in yield, the treated group declining to the level of the controls within some 10 days of the last injection. Fourteen days after the last injection all animals were dried off.

Intake Estimates

The intake of each animal, for each of the three weeks estimations were made, is recorded in Table 16. The digestibility estimates upon which the D.O.M. intake has been calculated are also included. Using maintenance requirements as calculated in Trial 1, the net efficiency of production of 4% milk for each animal has been derived. This estimate of net efficiency apparently breaks down in the case of twin sets 77, 78 and 267, 268. In each set the D.O.M. intake appears to be unduly low, though repeatable from week to week. No satisfactory explanation of this can be provided. With the other two sets, however, the efficiency

TABLE 10. MILK YIELD DURING THE TWO WEEKS PRIOR TO THE FIRST INJECTION (LB. MILK)

Twin Set	Week 1		Week 2	
	Growth Hormone	Control	Growth Hormone	Control
37–38	138.7	137.3	124.7	145.0
77–78	105.5	86.1	103.3	83.0
185–186	106.1	138.2	118.7	127.8
267–268	123.6	95.8	110.8	93.9
	473.9	457.4	457.5	449.7

TABLE 11. MILK YIELD DURING THE INJECTION PERIOD (LB. MILK)

Twin Set	Week 1		Week 2		Week 3		Week 4	
	Growth Hormone	Control	Growth Hormone	Control	Growth Hormone	Control	Growth Hormone	Control
37–38	161.0	143.7	153.3	117.0	160.5	112.0	126.8	93.1
77–78	130.5	87.9	132.7	82.6	132.8	77.4	108.3	62.6
185–186	156.9	144.2	171.0	138.8	170.7	140.4	148.6	119.2
267–268	150.3	100.1	165.2	78.1	173.0	84.6	132.2	71.9
	598.7	475.9	622.2	416.5	637.0	414.4	515.9	346.8

TABLE 12. MILK YIELD DURING THE FORTNIGHT SUBSEQUENT TO THE LAST INJECTION (LB. MILK)

Twin Set	Week 1		Week 2	
	Growth Hormone	Control	Growth Hormone	Control
37–38	101.1	103.0	68.6	94.6
77–78	93.0	65.4	70.3	52.3
185–186	106.9	108.0	80.1	90.1
267–268	92.4	68.4	77.2	64.8
	393.4	344.8	296.2	301.8

TABLE 13. BUTTERFAT YIELD DURING THE TWO WEEKS PRIOR TO THE FIRST INJECTION (LB. FAT)

Twin Set	Week 1		Week 2	
	Growth Hormone	Control	Growth Hormone	Control
37–38	6.49	8.10	8.10	6.81
77–78	5.80	4.44	5.78	4.25
185–186	6.98	9.30	6.68	7.48
267–268	6.72	4.86	5.83	4.88
	25.99	26.70	26.39	23.42

TABLE 14. BUTTERFAT YIELD DURING THE INJECTION PERIOD (LB. FAT)

Twin Set	Week 1		Week 2		Week 3		Week 4	
	Growth Hormone	Control	Growth Hormone	Control	Growth Hormone	Control	Growth Hormone	Control
37–38	7.92	7.32	7.66	5.54	7.24	5.69	6.75	5.26
77–78	6.50	4.67	8.97	4.59	7.36	3.89	7.92	3.75
185–186	8.28	7.32	8.17	8.86	8.82	7.77	9.36	6.17
267–268	6.98	4.95	8.93	4.44	9.34	3.99	8.38	3.96
	29.68	24.26	33.73	23.43	32.76	21.34	32.41	19.14

TABLE 15. BUTTERFAT YIELD DURING THE FORTNIGHT SUBSEQUENT TO THE LAST INJECTION (LB. FAT)

Twin Set	Week 1		Week 2	
	Growth Hormone	Control	Growth Hormone	Control
37–38	4.77	4.83	3.55	4.86
77–78	5.92	4.27	4.29	2.96
185–186	5.75	6.93	4.82	5.93
267–268	5.33	3.62	4.28	3.38
	21.77	19.65	16.94	17.13

TABLE 16. MILK YIELD, BLOOD SUGAR, AND FEED INTAKE (TRIAL 2)

Twin Set	Yield (lb. F.C.M.)	Intake (lb. D.O.M.)				Digestibility (%)				Live-weight (lb.)	Mntce. Requirement (lb.)	F.C.M. Net Intake	Treatment	Blood Sugar
		Week 1	Week 2	Week 3	Total	Week 1	Week 2	Week 3	Average					
37	532	164.9	151.7	143.7	460	79.2	81.0	80.8	80.3	970	237	2.38	G.	47.7
38	390	156.0	162.4	132.6	451	80.0	81.2	80.2	80.5	910	227	1.74	C.	46.0
77	512	113.1	97.1	78.4	289	79.7	81.3	80.2	80.4	930	231	8.88	G.	46.1
78	282	111.0	108.5	89.4	309	80.4	81.9	81.3	81.2	910	227	3.43	C.	41.8
185	602	142.8	154.8	142.7	440	81.0	81.7	81.2	81.3	940	233	2.91	G.	49.4
186	483	148.3	150.8	135.1	434	80.0	82.5	81.1	81.2	920	229	2.35	C.	45.6
267	552	116.0	120.6	100.4	337	80.0	81.6	81.5	81.0	890	225	4.92	G.	44.6
268	288	89.6	81.2	79.7	251	79.5	81.2	81.0	80.6	850	216	8.39	C.	47.4

D.O.M.—Digestible organic matter.

F.C.M.—Fat-corrected milk.

estimates compare favourably with those derived from Trial 1, and again suggest that the increased milk yields associated with growth-hormone therapy are accompanied by an increase in efficiency of milk secretion.

Blood Sugar

For convenience, the average weekly blood-sugar estimates have been included in Table 16. Slight rises were recorded in three animals, one animal showing a slightly lowered level, however.

DISCUSSION

A general belief countenanced by dairy husbandmen implies that techniques applied to lactating cattle with the object of increasing lactation yield hold the greatest chance of success if applied once the animals involved have passed their peak milk yield. Thus Petersen[8] states: "In all experiments of this kind it has been found that lactation increases are greatest when treatment is made in the declining phase of lactation." More recently, thyroid therapy has been recommended only when the animal is past the peak phase of secretion[9]. As a consequence, the idea has emerged that the secretion of anterior pituitary factors is not the general limiting factor to milk production at the peak phase of lactation[10]. Rather, there is a widely held belief that the intra-alveolar pressure of the mammary gland is at a maximum at the lactation peak and declines thereafter.

Judged in the light of these opinions, the more or less equal response of the animals injected with growth hormone at the peak of lactation compared with those injected in the declining phase of lactation is indeed surprising, especially in view of the magnitude of the response obtained. The validity of the above-mentioned ideas thus warrants closer examination. In reporting the results of an experiment of importance to this problem, Fawns *et al.*[10] comment as follows: "The interpretation of the results of experiments on animals at the peak of lactation is beset by the difficulty of obtaining satisfactory control data for any particular animal." The recent advent of the use of identical-twin cattle in animal experimentation certainly overcomes in a large measure the problem of satisfactory control animals. As more data from such critically controlled experiments become available, the relationship between the sensitivity of response to an imposed treatment and the stage of lactation of the animals involved may well be resolved. Of interest in this regard is some recent evidence obtained from lactating sows, implying that, in the sow at least, intra-alveolar pressure does not decline as lactation progresses but remains constant throughout the lactation period[11]. A realization of this may be of value in interpreting the results reported herein.

Concurrent with a greatly increased milk yield recorded in the growth-hormone group of animals, there has been an apparent increase in the net efficiency of the lactation process. This result may, however, be queried on the grounds of an induced variation in tissue structures between the growth-hormone and control groups. Even though there was no change in the relative body weight of the two groups, either during or after the therapy treatment, it is possible that in view of the known ability of growth hormone to catabolize fat and build protein, in both rats[12] and pigs (Ruakura data—unpublished), a similar tissue

change may have occurred in the cattle under discussion, thus providing endogenous energy. In this respect it is interesting to note that growth hormone was without effect on the growth rate of fully fed identical-twin calves. The final appraisal of this situation must, however, await accurate observations on the effect of growth hormone on the carcass composition of lactating dairy cattle. The fact that thyrotropic-hormone depressed the rate of gain of body weight whereas the same dosage, administered along with the growth hormone, produced no change from normal is worthy of note.

A particularly interesting feature of the lactational response to growth-hormone therapy has been the apparent lack of any induced endocrine homeostasis. Thus, with the cessation of injections, the milk yield of the growth-hormone group dropped slowly to the level of the controls and remained at about this level thereafter, whereas the thyrotropic-hormone and thyroxin group dropped well below the level of the control group. A similar pattern of behaviour was apparent for the heart-rate response. Whilst sustained daily therapy of growth hormone elevated the daily milk and fat yield, this increased yield remained in a fairly constant ratio to the yield of the control animals. In other words, the growth-hormone therapy alone appeared incapable of maintaining an animal at the peak of lactation for an indefinite period.

In view of the marked diabetogenic action of growth hormone in cats and dogs[13], the lack of any change in blood-sugar concentration attributable to the growth hormone, as such, is an interesting observation; likewise the absence of any marked variation in the other blood and milk constituents studied. This result would appear to suggest that the galactopoetic effect of growth hormone is not mediated by any obvious mass-action phenomena whereby the concentrations of blood metabolites of importance to milk synthesis are altered in any way. To imply that there was any clue whereby the increase in yield could be explained, however, would indeed be wishful thinking, for the only apparent physiological and morphological response of the cattle treated was to develop much larger mammary glands and to secrete considerably more milk. Since these results were obtained, some further data accumulated by the authors suggest that growth hormone has galactopoetic properties in the lactating rat but affects neither litter weight nor mammary development when administered daily to maiden does throughout the gestation period only.

In spite of the fact that there is no real knowledge of the reasons for the increased milk yield, it is possibly of value, and certainly of interest, to speculate on the physiological role that growth hormone may play in the normal mechanisms of lactation. There is available reasonable evidence indicating that three-times-a-day milking produces superior milk yields to twice-a-day milking[14], while a number of workers have increased the milk and fat yield of animals over comparatively short periods of time by removing the residual milk of the mammary gland[15, 16]. In such cases the increase in production has been usually attributed to a general reduction in the intra-alveolar pressure and, by inference, a consequent increase in the subsequent rate of milk secretion. Recent unpublished evidence from Ruakura suggests, however, that the decline in secretion rate per unit time is insufficient to account for the differences in production reported in such experiments. Thus, the possibility exists of the stimulus of milking being of greater importance

than merely causing the onset of milk ejection. Recently it has been demonstrated that oxytocin, which is probably the most important milk injection principle released in response to the milking stimulus, actually causes the release of A.C.T.H. and T.S.H. from the anterior pituitary[17 18], while recent observations at Ruakura suggest that the rate of milk secretion *per se* is increased by the administration of oxytocin. It thus seems feasible that the more frequent release of the milk ejection factors, associated with frequent milking, may in turn liberate anterior-lobe factors in greater quantity, amongst them the growth hormone. Thus may the end result of frequent milking possibly be explained.

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SEX HORMONE STUDIES ON SHEEP

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Summary

1. Investigations are described on the sex hormones of Romney sheep.

2. A means of continuously collecting urine from grazing ewes was devised, and all observations were made on samples collected under these conditions.

3. The chemical method of Pincus was modified to remove toxic *p*-cresol from extracts used for bioassay.

4. Investigation of 8 different chemical methods for extraction of oestrogen and androgen from ewes' urine showed that this modified method of Pincus was as efficient as any of the others tried for oestrogen extraction with one possible exception. For androgen extraction it appeared that some other methods might be more efficient, though likely differences were masked by lack of precision of both the bioassay and chemical methods. Hydrolysis of urine was done at refrigerator temperature for several weeks without any loss of oestrogenic or androgenic activity.

5. About half the oestrogenic activity in a bulked sample of late-pregnancy urine was in the free state. Chemical separation methods showed that the proportions of the different fractions in a twenty-four-hour sample of late-pregnancy urine were of the order: oestrone equivalent 4.5 μg; oestradiol equivalent 2.4 μg; oestriol equivalent 2.0 μg.

6. Observations made in the application of different bioassay methods showed that more work is desirable concerning the application to animal extracts of techniques developed on pure substances.

7. It was also shown that systematic studies are needed to determine the various non-hormonal components of sheeps' urine and the proportions of the different oestrogenic substances excreted in different sexual conditions.

8. No oestrogenic or androgenic activity was found in urine collected from rams shortly after the breeding season.

9. No oestrogenic activity was found in urine from anoestrous ewes. During the breeding season small amounts, up to 2.4 μg. equivalent oestrone in twenty-four hours, were detected. The amount at mid-cycle was as high as in urine from oestrous ewes.

10. In pregnant ewes, oestrogens were usually detected first in the urine at about the fifteenth week, and as early as the eighth week in one ewe. Androgens were first detected at about the same time. No oestrogens or androgens were found in lactating ewes' urine. The relationship of these findings to other physiological phenomena during gestation in the ewe is discussed.

11. Pregnant ewes grazing on oestrogen-potent red clover excreted appreciably more oestrogens in their urine than grass-fed animals.

12. No gonadotrophic activity was found in large samples (500 ml.) of blood collected at oestrus and other times of the cycle.

Introduction

As a basis to research on the reproductive performance of sheep, studies on the sex hormone status of this animal were initiated at Ruakura some five years ago.

The literature on the distribution of sex hormones in farm animals has recently been reviewed by Cowie[1] and Cole[2]. Very little information is available for sheep. Whitten[3] demonstrated the presence of very small amounts of oestrogens in urine during the last few weeks of pregnancy, and 17-ketosteroids at all stages. Beck[4] found small quantities of oestrogens in urine and faeces during the last three or four weeks of gestation. Neher and Zarrow[5] reported progesterone in the peripheral blood. More recently Edgar[6], using a chemical method, detected progesterone in the ovarian vein draining an active ovary in both pregnant and non-pregnant ewes, but not in peripheral blood. All attempts to find gonadotrophins in the body fluids have failed.

The difficulties in this type of work in sheep are associated with the fact that extremely small amounts of hormones are present. Consequently it is necessary to extract very large volumes of fluid. The methods for both extraction and bioassay for the different sex hormones have been developed on species—chiefly human—in which relatively large amounts are found.

The present paper presents the Ruakura results to date. Many of the findings are of a preliminary or suggestive nature only, and are published at this stage mainly so that other workers may profit by our experiences.

Collection of Material

Mature Romney Marsh sheep were used. Except in one instance, they were grazing freely on a pasture predominantly composed of perennial ryegrass and white clover.

An apparatus[7] was designed for making continuous collection of urine from ewes in the field. In the few cases when rams' urine was used, the apparatus designed by Sears[8] was adopted. Blood was cannulated from the jugular vein.

Chemical Methods

Total Oestrogens and Androgens in Urine

In view of the small amounts of biologically active material excreted, it was found necessary to extract the whole of a twenty-four-hour sample of urine. This volume varied considerably according to the time of year. Between half to one and a half litres was usual during the autumn period, while a preganant ewe on lush wet spring pasture voided as much as five litres.

In the first instance, the chemical method of Pincus[9] was chosen as the only one suitable for routine handling of such large volumes. It was necessary to use sodium tauroglycocholate to break the emulsions formed with ether in the initial extraction[10]. The final concentrates, however, proved so toxic to the test mice that bioassay was impossible. The toxin was identified as *p*-cresol, and effectively removed by saturation of the solution with sodium chloride, and steam distillation at the stage when the alkaline extract containing the oestrogens had been acidified[11]. The different stages of the modified method can thus be summarized briefly as follows:

Hydrolysis with HCl

Ether Extraction

Ether Extract Treated with 2N NaOH

Ether residue, containing androgens (neutral steroids) evaporated.	NaOH fraction, containing oestrogens, phenols etc. Acidified to pH 2 with 33% HCl and saturated with NaCl
	Steam Distilled
Volatile *p*-cresol and benzoic acid.	Oestrogens extracted by ether. Evaporated.

The amount of dry residue was usually about 0.2g. per litre of urine.

It was shown that small quantities of oestrogens and androgens, bioassayed by standard methods, could be removed by this procedure from suitable urine. However, it was thought that more efficient extraction might be possible. Several workers have compared various methods used for human urine. After extensive perusal of the many different methods described in the literature, seven representative types were selected to compare with that described above, hereafter referred to as the "standard" method.

In each case, a bulked sample of about six litres of urine was collected during late pregnancy, previous experiments having shown the highest concentration of oestrogens and androgens at this time. This volume was divided into three equal parts. The standard method was used for one portion, and the others extracted in duplicate by two different workers, using the alternative methods.

The essential features of the various processes are summarized and results presented in Table 1, and reference is made to typical papers in which similar methods were used for human urine. The chief differences were in the hydrolysis procedure, concentration of hydrochloric acid, time, and temperature (methods 2–6). Except for method 5, all solutions were boiled during hydrolysis. For each comparison, bioassays on the three portions were made simultaneously. The spayed mouse vaginal smear technique[12] was used for oestrogens, and inunction of the capon's comb[13] for androgens. Results of the assays showed that the standard method was quite as efficient in the extraction of oestrogens as any of the methods 2–8. Variations in hydrolysis procedure appeared to have little effect. Even urine acidified and allowed to stand in the refrigerator over a long period did not lose biological activity. There was very close agreement between the standard and the two duplicates in all cases. Method 8 appeared more efficient, but the laborious procedure involved precluded its use with large volumes and

TABLE 1. CHEMICAL METHODS—VARIATIONS OF PROCEDURE

No.	Per Cent HCl	Hydrolysis Time	Temperature and Conditions of Extraction	Typical Reference	Oestrone Equivalent (μg per 1,000 ml)
1	5	10 min.	Routine method	(9)	1.21, 1.08, 0.83
2	2	30 min.	Routine method, different conditions	—	0.87* 0.87, 0.87
3	5	60 min.	Routine method, different conditions	—	0.84, 0.84, 0.88
4	5	2 hours	Autoclave 20 lb. per sq. in.; extracted as in 1.	(27)	0.78, 0.84, 0.78
5	To pH 0.6	4 weeks	At 4°c. in dark, extracted as in 1.	(28)	2.12, 2.12, 2.20
6	15	2 hours	Refluxed with zinc dust, extracted as in 1.	(29)	1.50, 1.90, 1.65
7	To pH 6	—	Extracted 3 times with *n*-butanol to extract conjugates. Butanol evaporated and aqueous solution hydrolysed and extracted as in 1.	(30)	1.20, 0.72, 0.72
8	5	10 min.	As in 1, but ether extract concentrated, diluted with 18 vols. CCl_4 before sodium hydroxide separation of oestrogens and androgens	(14)	1.20, 1.52, 1.45

*First line is value from routine method, second line gives values for the new procedure, done in duplicate.

numbers of samples. Results of the androgen extraction experiments were a great deal less clear-cut. One of the chief weaknesses lay in the assay method, which was not so precise as the one used for oestrogens. There appeared to be a lack of agreement between duplicates, which, though partly masked by imperfection of the assay technique, suggested that chemical extraction of androgens is not so reliable as for oestrogens.

Oestrogen Separation

In two experiments separation methods, using Girard's reagent (Friedgood[14]), were used to determine the proportions of the different oestrogenic components in pregnant ewes' urine. Two (24 h.) samples were taken from each of two ewes in late pregnancy. The resulting extracts were assayed by the vaginal smear method, against the appropriate standards.

The proportion in the different fractions in a twenty-four-hour sample was of the order: oestrone equivalent 4.5μg; oestradiol equivalent 2.4μg; oestriol equivalent 2.0μg; though there was some variation between the samples.

Free Oestrogens

A bulked sample of late-pregnancy urine was divided into three equal portions of 1,700 ml. One part was extracted by the standard method. The other two parts were treated as duplicates. Urine was first extracted without hydrolysis to remove any free hormones. The residual urine was then hydrolysed and extracted to obtain the combined hormones. Assay results showed that approximately half the total oestrogen was present in a free state.

Gonadotrophins in Blood

For extraction of gonadotrophins from blood, the methods of both Cartland and Nelson[15] and Rimington and Rowland[16] were tried. Samples of about 500 ml. of citrated whole blood, when centrifuged, yielded between 50% and 70% of serum. Final precipitates were made up to 30 ml. with 0.9% sodium chloride, for injection into the test animals. It was found that the first method was more convenient to use. The second one was very lengthy and sometimes yielded so much final precipitate that great difficulty was experienced in injecting the rats.

BIOASSAYS

Urinary Oestrogens

Both the spayed mouse vaginal smear method[12] and the infantile mouse uterine weight method[17] were used extensively on urine extracts. The more sensitive intravaginal technique (Emmens[18]) has also been considered.

For subcutaneous injection, the extracts were taken up in peanut oil. The dried extracts were dissolved in a few ml. of alcohol and 4 ml. of peanut oil were added. The alcohol was removed on the water bath *in vacuo* and the oil solution made up to 10 ml., or later, when it was found that such small amounts of oestrogen were present, to 6 ml. with oil. Thus, for a three-litre sample a spayed mouse would receive the equivalent of 100 ml. of urine.

Oestrogenic activity could be demonstrated in ewes' urine, in certain conditions, by either of the first two methods. Frequently a response could only just be obtained, on a sufficient number of mice, with the whole extract from a twenty-four-hour sample. Hence, a slight change in sensitivity of the test animals might result in failure to detect any activity at all, and it was usually not possible to test any sample at more than one dose level, thus reducing the accuracy of such assays.

The spayed mouse (subcutaneous) method was used in the early stages of the work on pregnant ewes and for the experiments on chemical methods. During one period, symptoms typical of vitamin-A deficiency appeared in the spayed mice, including cornification of the vaginal epithelium of uninjected animals. Use of such mice for oestrogen assays would, of course, lead to false positive reactions. A change in the diet remedied this condition. Even then it was found that from time to time large numbers of nucleated cells would appear in the check smears from untreated mice, and the condition would clear up as suddenly as it appeared. The reason for this was obscure. However, it did add to the disadvantages of this method.

During the period of dietary deficiency in spayed mice, many urine samples were collected from ewes during the breeding season.

To determine whether there was any oestrogenic activity at all in the samples (and hence whether it was worth continuing with the experiment), the immature mouse uterine weight bioassay method[17] was adopted. Quite marked responses were obtained in many cases. Several of these same samples were subsequently tested by the vaginal smear (subcutaneous) technique[12] and little or no response obtained, though when re-checked by the uterine weight method it was apparent that no loss of activity had occurred. For example, an extract tested by the former method gave a mean uterine weight (10 mice) nearly three times that of the controls, equivalent to 0.175 μg. oestrone; by the latter method only a 10% response was obtained, equivalent to 0.05 μg. oestrone. Thus if oestrone is used as a standard in both cases, by the first method (0.3 ml. total dose) the amount present in the whole sample would be equivalent to 3.5 μg. oestrone, but by the second equivalent to only 1.5 μg.

A few samples of pregnancy urine were tested by both methods and the difference in sensitivity of the two methods was less marked.

In view of these difficulties it seemed that the most suitable method was that involving direct intravaginal application of the test substance. Emmens[18] has shown this to be about two hundred times more sensitive than the subcutaneous technique for oestrone, and the difference in relative potency of the different oestrogens less marked. The chief problem was to find a suitable vehicle in which to apply the test material. The weight of the final urine extract was considerable and was composed mainly of tarry non-hormonal substances that are present in sheeps' urine. The actual amount of oestrone equivalent in a twenty-four-hour sample of late pregnancy urine thus represented about 1 part in 100,000 of dried residue. A solution in glycerol-water (1:1), with the test material taken up in no more than 5% alcohol, has been used successfully with pure oestrogens. However, the urine concentrates would not go into solution or suspension in the glycerol-water containing only 5% of alcohol. Higher concentrations of alcohol had an adverse effect on the vaginal surface, producing numerous nucleated cells.

Several aqueous dispersing agents that were tried made successful suspensions of the urine residues but caused vaginal cornification when applied alone. These included non-ionic detergents, 1% soap solution, and carboxymethylcellulose. The most promising substance was 0.3% solution of agar. The urine extract was dissolved in acetone then mixed with the required amount of agar solution. If this mixture was heated until all the acetone had been removed, the urine concentrate was evenly dispersed through the agar. Difficulty was experienced in causing all of the extract in a sample to go into a sufficiently small volume of agar. Agar solution alone had no effect on the vaginal epithelium. Investigations were not continued beyond this stage.

Augmenting substances. Some observations suggested that augmenting substances might be present. Emmens[12] has described the presence of such material in human urine. Benzoic acid has been shown to have such an effect. During the investigations on *p*-cresol it was shown that the bulk of this substance was driven off during steam distillation, but some may have remained in the residue.

Stability of extracts. The oestrone standard was made up in a concentration of approximately 10 mg. oestrone in 25 ml. peanut oil, and diluted at each test to the required strength. Oily solutions of oestrogens are generally supposed to be stable. However, the concentrated solution lost some of its activity after about six months at room temperature on at least three occasions. For example, a standard made up so that each spayed mouse received 0.2 μg. oestrone caused a 100% response when fresh, but in ten months no response was obtained. This was not due to a marked change in sensitivity of the test mice since a freshly prepared solution of oestrone of the same strength tested at the same time gave 100% response. Oestradiol and oestriol solutions appeared to be relatively more stable. Sheep extracts in oil seemed to be of variable stability. Some samples gave approximately the same response after two years at room temperature, while others appeared to lose activity even when stored as dried material.

Urinary Androgens

The total amount of androgen in any extract did not exceed 250 μg., the amount usually being rather less, so that a relatively sensitive method was required. The chick comb method (Frank[19]) was used for a few samples in the first season, the extract in oil being applied to the comb. Though this proved sufficiently sensitive for such small amounts, the method was inconvenient. Commercial chick hatching in New Zealand takes place over a limited period of about three months in the spring. With no facilities for poultry-breeding at Ruakura, it was not considered worth going to the expense of establishing these if an alternative method could be used. Accordingly, the capon comb method[13] was used in all work recorded in this paper, the extract again being directly applied to the comb. The birds used were white leghorns, bought in as day-old chicks and caponized at 6 weeks. There was great variability in response between birds. Each androgen fraction from the chemical methods experiment was assayed on 5 birds, and the three fractions from each method were assayed simultaneously. Each assay was repeated on an additional 5 birds between one and three months later. Disagreement between duplicates of each method and reversal of results on repetition indicated that the bioassay technique was not adequate to detect differences between the methods of extraction. There was much less variation in response to standard androsterone on different occasions than there was with the crude extracts. Results from the methods experiment, calculated in terms of androsterone, showed values from 20 to 200 μg. per litre.

EXPERIMENTS WITH SHEEP

Oestrogen Excreted by Non-pregnant Ewes

Anoestrus. (Uterine Weight Assay). Samples from non-pregnant ewes were taken during the months November to January. Only one collection was made from each of two of these, and two collections at an interval of a fortnight from the other animals, towards the end of January. Four of the tests were entirely negative. Two extracts from the same ewe on 16 and 30 January gave a doubtful response. Single samples from two hoggets, collected at the same time of the year, gave no response. No collections were made during February.

Oestrus and Dioestrus (Breeding Season). (Uterine Weight Assay). Six ewes were used in an experiment to determine the oestrogen excretion

TABLE 2. OESTROGEN EXCRETION DURING THE OESTRUS CYCLE

	Heat	2–8 Days	9–16 Days
No. of observations	13	4	8
No. of negative tests	2	1	1
μg. equivalent oestrone in 24 hours	1.1 – 2.4	1.1 – 1.2	1.3 – 2.4

at different stages of the oestrus cycle (Table 2). Three were well flushed and three run on very short, hard pasture. A period of six weeks was covered (March to mid April). Collections were made from each ewe for each observed heat. Three heats were recorded for three animals, while for the remainder only two heats were observed. Collections were also made at several different times during dioestrus.

There was no obvious difference at comparable stages between the excretion of flushed and unflushed ewes, or between successive heats as the season advanced. Altogether thirteen collections were made during heat. In two cases no biological activity was found. Eleven extracts gave positive responses, equivalent to a range of 1.1 to 2.4 μg. oestrone, for a twenty-four-hour elimination of urine. Four collections were taken in the first half of the cycle, from the 5th to the 8th day. There was no response to one of these and the other three gave results equivalent to 0.9 to 1.4 μg. oestrone. Nine collections were made, at different times, from the 9th day of the cycle until two days before the next heat. Values from eight of these assays per twenty-four hours varied from 1.3 to 2.4 μg. equivalents of oestrone. The highest response was from a sample collected on the 10th day. One extract gave a negative response.

Thus there was no obvious difference between the excretion during heat and during the second half of the cycle. Though values during the first part of the cycle appeared to be lower, there were not enough observations for any conclusion to be drawn.

Oestrogen Excretion by Pregnant Ewes (Vaginal Smear Assay)

Weekly urine collections were made through pregnancy, in two different seasons; observations were made on three ewes for one year and one ewe for two years (Table 3).

There was considerable variation between ewes both in the quantity excreted and the time at which detectable amounts appeared in the urine. For one ewe the level of excretion began to rise at the eighth week and remained approximately the same each week until the 15th week when it rose to 5.1 μg. of equivalent oestrone; in the 18th week it had risen to 20 μg. In the 19th week it had fallen to 9 μg. This animal died of pneumonia two weeks before her lambing date. A sample taken in the period immediately before death contained only traces of activity. No traces of oestrogen were found in the urine of another ewe until the 18th week. The amount present was approximately the same in the

TABLE 3. OESTROGEN EXCRETION BY PREGNANT EWES
(μg. equivalent oestrone per 24 h.)

Weeks Pregnant	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Post-Lambing
Ewe No. 1							—	—	—	—	T	2.5	3.0	6.0*	1.5
„ „ 2					—	—	—	2.0	1.5	2.5	2.1	3.2	3.0	*T	T
„ „ 2				—	T	T	T	1.8	1.5	3.0	1.5	T	4.1	5.6†	
„ „ 3	1.8	T	2.1	2.1	1.5	1.2	T	5.1	5.1	20	20	9.0	Died T		

* 36 to 12 hr. before lambing
† two days before lambing
T traces

19th and 20th weeks (3.0 μ.g) and rose to 6.0 μg. in the period 36 to 12 h. before lambing. There was some activity in the urine during the period 12 to 36 h. after lambing, but after that all traces had disappeared. Ewe No. 2 followed much the same pattern of excretion in both seasons. Collections were made from two of the ewes immediately before lambing; in one the level had dropped but in the other it was higher than in the previous collection.

Oestrogen Excretion by Special Ewes

Ewes grazing on red clover. The excretion of oestrogens in urine of ewes grazing red clover was studied. At the beginning of the experiment the clover was in a leafy pre-flowering stage and had an oestrogenic activity equivalent to 0.06 μg. oestrone per g. clover (wet weight). Five normal ewes and one spayed ewe were run, with a fertile ram, on the area for two months during the tupping season. At the end of this time, when all the entire ewes were in lamb (between 5 and 7 weeks), twenty-four-hour urine collections were made from each animal and were extracted and assayed by the uterine weight method. Mean uterine weights of approximately twice those of untreated mice were obtained in all tests, giving values equivalent to 1.4 to 1.8 μg. oestrone. Urine extracts from two grass-fed control ewes in comparable stages of pregnancy gave no response, while for a third animal a doubtful response was obtained. Oestrogenic activity equivalent to 1.2 μg. oestrone was found in the urine of the clover-fed spayed ewe, whereas there was none in the urine collected from a grass-fed spayed ewe.

No actual measurement of the amount of clover eaten by each ewe was made. However, this was estimated at about 10 lb. wet weight per day, which would include about 270 μg. oestrone equivalent. It thus excreted in the urine about 0.6% of the amount ingested.

Ewe implanted subcutaneously with 1 g. tablet of stilboestrol. Samples were collected from this ewe a few days after implantation of the tablet. In this case urine was obtained by suppression of the animal's breathing. This is a very effective technique if small amounts of urine are required. Five extractions were made, on volumes varying from 290 to 858 ml. Assays were done by the vaginal smear method, using stilboestrol as a standard. Two extracts, from volumes of 340 and 375 ml., were negative. The other three gave values equivalent to between 1.4 and 4 μg. stilboestrol per 100 ml. of urine.

TABLE 4. ANDROGEN EXCRETION BY PREGNANT EWES

Weeks Pregnant	6	9	12	15	18	21
No. of observations	1	7	11	11	11	7
No. negative tests	1	1	3	2	1	
Traces		4	7	3	T	
Reactions		2	1	6	9	7
μg. equivalent androsterone per 24 hours		34–52	54	42–200	25–220	30–250

T = Traces

As a ewe at this time of the year would void about 2,000 ml. of urine in twenty-four hours, the total excretion during this period could be estimated at 80 μg. equivalent of stilboestrol, or 0.008% of the implanted tablet.

Androgen Excretion by Pregnant Ewes

Assays for androgenic activity were done on urine extracts from eleven different ewes at different stages of gestation (Table 4). As with oestrogen excretion, there was much individual variation. For two ewes no androgenic activity was found in any sample until the 21st week of pregnancy, while for a third ewe appreciable amounts were first observed at nine weeks.

The majority of animals excreted very slight traces at twelve weeks, and appreciable amounts in later stages. There was no relationship between androgen excretion and/or number of foetuses carried.

Hormone Excretion in Rams' Urine. Twenty-four-hour urine samples were collected from each of six rams in July, two months after the end of the mating season. Three animals were fertile and three were suffering from epidydimitis. No androgenic or oestrogenic activity was found in any sample.

Gonadotrophins in Blood. An attempt was made to demonstrate the presence of gonadotrophic hormone in the peripheral blood of ewes during the breeding season. Collections were made from the same ewes and at the same times as the urine collections for oestrogen extraction. Bioassays were done by the immature rat weight of ovary method[20]. Each rat received the equivalent of about 42 ml. of whole blood. No positive tests were obtained from any samples taken at heat or at other times in the cycle.

DISCUSSION

Several useful advances in technique have been made in the course of these investigations. The urine-collecting apparatus has made possible the study of ewes under natural grazing conditions. Previous work was done on animals confined in metabolism cages (Whitten[3]). Concentrated extracts of the large volumes collected under field conditions were found to contain much *p*-cresol. Bioassay of these samples would

have been impossible had this toxin not been identified and the extraction modified so that its removal was ensured. Campbell and Hey[21] have reviewed the literature on the occurrence of *p*-cresol in animal urine. They correlated the excretion of *p*-cresol with that of the oestrogenic hormones and suggested the possibility that the oestrone and *p*-cresol may be derived from the male hormone or some related steroid. However, in the investigations described above the *p*-cresol concentration was equally high in urines which had no oestrogenic or androgenic activity. In this connection it is worth recording the observations of one of us (O.K.S.) on four litres of urine from a cow with cystic ovaries. No oestrogens were detected in the extract, but approximately 0.1% of *p*-cresol was obtained by steam distillation.

Other valuable information from the work on chemical methods was that urine may be hydrolysed for several weeks at refrigerator temperature without loss of oestrogenic or androgenic activity, a finding which may greatly facilitate arrangements during rush periods.

There is need for further study on several aspects of both chemical and biological methods. More systematic work is required to identify the various non-hormonal components of sheeps' urine. Such unknown substances could be the source of much error in bioassay by affecting the stability of extracts under certain conditions, or by augmenting the biological activity of the hormones. Cohen and Bates[22] recently described a non-oestrogenic uterine-stimulating and oestrogen-augmenting substance in pregnant mares' urine. Such knowledge is also necessary for sheep if further purification of extracts is contemplated.

It is certain that, in any investigation such as this, much consideration should be given to the choice of assay method. Most bioassay techniques are developed using pure substances, and though they may be very reliable and repeatable under these conditions, many problems may arise when they are applied to animal extracts. This is well illustrated in the anomalous results from the use of the two oestrogen assays. In this instance further work is required to determine whether the relative proportion of the different excreted oestrogens varies in different physiological conditions. This is not unlikely if the oestrogens in pregnant ewes are produced largely by the placenta, and in non-pregnant ewes by the ovary. Also the metabolism of the oestrogen secreted may vary under different conditions, thus altering the identity of the excreted products.

More attention should be given to the application of the more sensitive intravaginal method for oestrogens. In choosing a vehicle for application of extracts it must be borne in mind that the slope of the dose-response line may be influenced by the solvent used (Biggers[23]). One of the disadvantages in the use of this method lies in the extreme sensitivity of the mouse vagina. It is possible that some of the impurities in the urine extracts could have an irritating action on the vaginal epithelium which would simulate a positive oestrogenic response.

Ideally, of course, a chemical assay method is most desirable. Edgar[24] has developed a paper chromatographic technique for blood progesterone, and it appears likely that a similar method could be used for blood oestrogens, if a sufficiently high concentration is present. A great deal of purification would be necessary before such a method could be used on urine extracts, and then it is likely that the amounts present in a twenty-four-hour sample would be too small to be detected.

Whitten[3] used a colorimetric method for determination of 17-ketosteroids. Such methods were found to be unreliable on androgenic extracts from these grazing sheep, because of the presence of large quantities of highly coloured impurities.

As far as the hormone excretion of sheep in different conditions is concerned, it seems now fairly well established that the urinary oestrogen rises towards the end of pregnancy. Both Whitten[3] and Beck[4] reported the presence of oestrogens during the last month only. In these studies oestrogens were detected as early as eight weeks in one animal, though about the fifteenth to seventeenth week appeared to be more typical. The level of androgen excretion rose at about the same stage or slightly earlier, though as relatively much larger amounts were excreted it was probably easier to detect slight traces in the urine. Relaxation of the sacro-iliac joints of the pelves of pregnant ewes which begins shortly before the twelfth week has recently been shown to be under hormonal control[31]. It seems likely that all these phenomena are related to the fact that cotyledonary attachment of the placenta is not complete until approximately the seventy-eighth day (Assheton[25]).

The fact that there is no difference in oestrogen excretion between heat and other times of the cycle suggests that in the sheep the level of oestrogen remains fairly constant throughout the breeding season. The occurrence of heat must depend on change in secretion of other hormones involved. Edgar[20] has found a sudden fall of progesterone in blood from the ovarian vein, on the 17th day of the cycle.

In the mare, various workers have reported that oestrogen in urine is greatest at oestrus. In non-pregnant sows oestrogens have been found in the blood only during oestrus. Little work has been done on the oestrogens of non-pregnant cows (Cowie[1]).

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EFFECT OF SYNTHETIC SOIL CONDITIONERS ON PLANT NUTRIENT UPTAKE

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Summary

Analyses of mustard samples from pot experiments indicate that improved structure resulting from the use of soil conditioners may have little influence on nutrient concentrations in plant tissues, even where total nutrient uptake is greatly increased as a result of marked yield responses. A big increase in potassium concentration was found in plants on a potassium-deficient soil which received potash fertilizer and which showed large yield responses to a polyacrylonitrile conditioner, but other differences were smaller or non-significant.

Introduction

In the interpretation of results of plant analyses it is clearly important to have information on the effect of adverse soil structure on plant nutrient levels. In conducting pot experiments with soil conditioners it is desirable that the watering procedure be at least as severe on the soil as the natural rainfall. Also it is advisable to use a plant that does not protect the soil too much from the impact of raindrops. Mustard was grown and more than adequate water was supplied through exposure of the pots to rainfall. The pot experiments were conducted by H. A. Browning and T. F. Southon[1] with four problem soils of poor structure, with the following values for cation-exchange capacity and exchangeable bases:

Experimental Method

Four pots of each treatment were used in each of the four experiments, which were laid down in May 1953. "Krilium" of formulation number 6 was mixed with each soil at a concentration of 0.1%, "Flotal" at 0.2%. As the latter contains 2.6% soluble nitrogen, the equivalent amount of sulphate of ammonia was applied to the Krilium pots in experiments with soils 1, 3, and 4. In experiments 1 and 3, a mixture of ferric alum and peat, with a comparable formulation to Flotal, was also used. No fertilizers were used with soil 2. In experiment 1, the soil was limed to 80% saturation and potassium chloride applied in solution at 2 cwt/acre, plus trace elements, but no phosphate. In experiment 3, an additional application of sulphate of ammonia equivalent to 1½ cwt. per acre was applied to all pots 10 weeks after planting. In experiment 4, 3 cwt. superphosphate per acre was applied 10 weeks after sowing. Plants were harvested after 10 weeks growth in experiments 1 and 2, 17 weeks in 3, and 12 weeks in 4.

Samples analysed consisted of whole above-ground tissues at harvest. Nitrogen and phosphorus were determined chemically; potassium, sodium, and calcium by flame photometry; magnesium and manganese by spectrographic methods. The flame calcium determinations required the prior removal of aluminium and phosphorus.

Results

Mean yields of dry matter at 100°C., expressed as grammes per pot, are shown in the table, together with mean concentrations of nutrients shown as percentage of dry matter.

TABLE 1. SOIL ANALYSES

Soil	pH	CEC	Ca	Mg	K	Na	% Saturation
Wharekohe silt loam	4.6	9.2	2.9	1.1	0.25	0.44	51
Waiareka complex	5.8	45.8	18.9	10.9	3.13	0.46	73
Ahuriri lagoon	8.2		>10		VH		
Haur ki clay	5.3	27.8	10.9	13.9	0.75	0.26	95

Yield Responses. In experiments 1 and 2 responses to Krilium were large and significant at the 1% level, in 3 at the 5% level, while in 4 there was a small depression in yield significant at the 5% level. There were no significant responses to Flotal or ferric alum. The high standard errors in the first two experiments were probably due to the fact that the pots were overwet for long intervals and this had particularly adverse effects on uniformity of growth in the pots without Krilium.

Physical Properties. Results of aggregate analyses by P. F. Noble, as determined by wet sieving, have been reported elsewhere[1]. In the first three experiments, drainage and soil structure were improved with Krilium treatment, but not with Flotal. In experiment 4, though drainage was improved and the surface crust was not as hard, differences in aggregates as determined at the end of the experiment were small and inconsistent.

Plant Nutrient Levels. In experiment 1, there was a highly significant increase in K concentration as a result of the Krilium treatments. Flotal gave a significant decrease in the Na level. The apparent increase in P levels by Krilium, and decrease by Flotal, did not reach significance at the 5% level. There were no significant effects of Krilium on concentration of nutrients in experiment 2, owing to high variability of the analytical figures. There was a trend towards lower concentrations of N, Na, Mg, Ca, and Mn in plants from the Krilium treatments, suggesting a dilution effect with increased yields, but average P and K figures were higher in the Krilium plants. In experiment 3, Mg concentration was significantly depressed by Flotal, and Na by both soil conditioners. In experiment 4, the increase in Mn was significant with Krilium and highly significant with Flotal. The Flotal effect, however, appears to be unrelated to structure as this conditioner failed to improve drainage or water-stable aggregates.

The only large effect associated with yield increases was that of Krilium on potassium levels on Wharekohe silt loam. Only Krilium gave satisfactory growth and it is possible that the greatly improved structure may have allowed the plant roots to penetrate throughout the mass of the pot soil and recover more of the applied potash. This received confirmation from the soil tests as the Krilium-treated soil showed lower soil K tests than the untreated. This was the only trial in which potash was applied and the only trial in which such a marked difference in potassium concentration was noted. There is other evidence, such as that of Lawton[2], that uptake of some nutrients, especially potassium, may be considerably increased by improved aeration.

TABLE 2. YIELDS OF DRY MATTER AND CONCENTRATIONS OF NUTRIENTS

Treatments	Mean Yield (g.)	Mean per cent of the dry matter						
		N	P	K	Na	Mg	Ca	Mn
(1) *Wharekohe silt loam*								
Control	0.70	2.28	0.233	2.00	0.60	0.26	1.18	.0039
N	0.63	2.73	0.273	1.95	0.77	0.24	1.17	.0048
Krilium + N	2.20	2.79	0.326	3.72	0.92	0.28	1.20	.0032
Flotal	0.78	2.92	0.219	1.95	0.50	0.21	1.17	.0033
Ferric alum	0.80	3.12	0.268	2.19	0.53	0.24	1.25	.0041
Sig. diff. (5%)	0.3	NS	0.066	0.61	0.17	NS	NS	NS
(1%)	0.4	NS	NS	0.85	0.23	NS	NS	NS
C.V.*	20%	18%	16%	18%	16%	13%	11%	27%
(2) *Waiareka complex*								
Control	0.78	2.62	0.383	3.25	0.20	0.68	1.53	.0075
Krilium	2.55	1.99	0.451	4.08	0.13	0.50	1.23	.0036
Sig. diff. (5%)	1.36	NS	NS	NS	NS	NS	NS	NS
C.V.*	29%	13%	20%	14%	30%	19%	22%	35%
(3) *Ahuriri Lagoon*								
Control	1.63	2.42	0.244	1.92	1.19	0.52	1.69	.0025
N	2.53	1.93	0.200	1.98	0.79	0.46	1.52	.0013
Krilium + N	2.93	1.80	0.197	2.02	0.68	0.41	1.38	.0011
Flotal	2.53	1.86	0.196	2.25	0.58	0.34	1.59	.0012
Ferric alum	2.18	1.96	0.192	2.16	0.66	0.40	1.46	.0017
Sig. diff. (5%)	0.31	0.29	0.022	NS	0.11	0.08	NS	.0005
(1%)	0.44	0.40	0.031	NS	0.15	0.12	NS	.0007
C.V.*	8.5%	9.3%	6.9%	7.3%	9.1%	12.5%	13.0%	20.3%
(4) *Hauraki clay*								
N	2.80	1.78	0.376	1.44	0.30	0.69	1.17	.0066
Krilium + N	2.33	1.94	0.404	1.64	0.34	0.69	1.09	.0097
Flotal	2.52	1.98	0.370	1.42	0.29	0.75	1.26	.0220
Sig. diff. (5%)	0.33	NS	NS	0.19	NS	NS	0.12	.0035
(1%)	NS	NS	NS	NS	NS	NS	NS	.0050
C.V.*	10.7%	9.5%	5.5%	8.1%	15.5%	8.6%	5.7%	17.0%

*Coefficient of variation, i.e. standard error per observation as percentage of the mean value.

In general, where nutrient levels were low, the mean values suggested a trend towards lower levels of the deficient nutrient in the plants showing the greatest growth, for example nitrogen in experiments 2 and 3, and phosphorus and manganese in experiment 3, but the differences did not reach significance. Mean phosphorus concentrations were greater in Krilium-treated plants in experiments 1, 2, and 4 where

phosphorus was adequate, but here again significance was not established. In the experiments that showed large Krilium responses, the absolute uptake of most nutrients was of course considerably higher in the Krilium plants.

Bould and Tolhurst[3] in a pot experiment with rye adequately supplied with phosphorus, found no significant effects of Krilium on yield or on plant uptake of phosphorus and potassium, but in a rye-seedling experiment on the same soil there were small but significant increases in phosphorus concentration and total uptake. Murdock and Seay[4] found a similar effect with P^{32} labelled superphosphate, the conditioner slightly increasing the phosphorus content but not the crop yield.

Acknowledgements

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SCREENING TESTS WITH INSECTICIDES FOR CONTROL OF RED SPIDER-MITE (*Tetranychus urticae* Koch) ON BEANS

By H. JACKS, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(Received for publication, 24 September 1954)

Summary

Forty-one insecticides were tested in the glasshouse for control of *Tetranychus urticae* Koch on beans.

Effective substances were classified according to the following properties.

Contact effect: Materials based on mipafox, TEPP, malathon, and butylphenoxyisopropyl chloroethyl sulphite.

Residual effect: Material based on chlorparacide.

Contact and residual effect: Materials based on ethyl p-nitrophenyl benzenethiophosphonate, parathion, dimefox, schradan, petroleum oil, and demeton.

Other test materials were not as effective as the above.

INTRODUCTION

The red spider-mite is a serious disease of dwarf and runner beans (*Phaseolus vulgaris* L.) grown in glasshouses and out of doors. Screening tests with insecticides using this mite as test organism, were carried out as part of this Division's certification scheme and results are reported herein.

MATERIALS USED

Chlorinated hydrocarbons: Aldrin em. (23%) liquid; Aldrin w.p. (25%) wettable powder; Chlordane em. (50%) liquid; Chlordane w.p. (50%) wettable powder; and Lindane 25 (25%) wettable powder.

Organic phosphorus compounds: EPN-300 (27% ethyl p-nitrophenyl benzenethiophosphonate) liquid; Folidol (20% parathion) liquid; Fosferno 20 (20% parathion) liquid; Murphos 20 (20% parathion) liquid; Thiophos (15% parathion) wettable powder; Metacide (24.5% methyl parathion plus 6.2% parathion) liquid; Hanane (50% dimefox) liquid; Isopestox (90% mipafox) powder; Malathion (50% malathon) liquid; Murphy Tepp (40% TEPP) liquid; Pestox 3 (55% schradan) liquid; Sytam (70% schradan) liquid; Tetrax (70% schradan) liquid; and Systox (50% demeton) liquid.

Miscellaneous compounds: Aramite (15% butylphenoxy isopropyl chloroethyl sulphite) wettable powder; Arathane (25% dinitro capryl phenyl crotonate) wettable powder; Azobenzene w.p. (40%) wettable powder; Mitosan A (20% azobenzene) liquid; Mitosan B (20% azobenzene plus 16.5% DDT) liquid; YF 2816 (21% azobenzene) liquid; Colsul 40 (40% colloidal sulphur) paste; DPS (technical diphenyl sulphone) powder; Dieldrin em. (24%) liquid; Dieldrin w.p. (25%) wettable powder; Summer oil (80% petroleum oil, viscosity 75 (Red. 1. 100°F.) unsulphonated residues 95%) liquid; lime sulphur 15% polysulphides) liquid; Marlate (44% methoxychlor) wettable powder; Murtox (50% toxaphene) wettable powder; nicotine sulphate

(40%) liquid; Chlorocide (20% chlorparacide) liquid; Kloromite (45% p-chlorophenyl p-chlorobenzene sulphonate) wettable powder; Murvesco (50% p-chlorophenyl benzene sulphonate) wettable powder; Ovamite (50% p-clorophenyl p-chlorobenzene sulphonate) wettable powder; Ovotran (50% p-chlorophenyl p-chlorobenzene sulphonate) wettable powder; PCPPCBS (technical p-chlorophenyl p-chlorobenzene sulphonate) powder; and Sulphenone (48% p-chlorophenyl phenyl sulphone) wettable powder.

EXPERIMENTAL METHOD

French beans of the variety Black Prince (syn. Black Valentine) growing singly in 6-in. pots were used in these tests. The growing tip of each plant was removed as soon as the first pair of leaves had developed. Plants were tray watered.

Ten adult mites were placed on each leaf with the aid of a fine brush two to three days before application of insecticides.

Five plants per treatment were used in Trial 1, and four plants in Trial 2. The surface of the soil was covered when systemic insecticides were applied to prevent intake by roots thereby increasing concentrations beyond that of foliage applications. Each plant was sprayed with 80-100 ml. of insecticidal suspension, a quantity found to be sufficient to allow thorough wetting of four to five two-leaved bean plants. Check plants were sprayed with 80 to 100 ml. of water. After drying, plants were moved to a glasshouse held at 71° ± 7°F.

In Trial 1, mite counts were made at four and 12 days after spraying; in Trial 2, at one and 12 days after spraying. The first count was made before mites could have hatched from eggs laid subsequent to infestation. In the second count, however, some mites which had hatched after spraying were included. Initial control and any residual effect were thus assessed from counts of active mites, but eggs were not counted. It is known that some of the materials used have ovicidal properties, while others become systemic poisons, and it is also possible that newly hatched mites may be killed by chemical residues on leaves. The phrase "residual effect" is used to cover all forms of acaricide action other than direct contact effects on active mites.

Treatments causing plant damage in Trial 1 were excluded from further tests.

Chemicals used in Trial 1 (Table 1) that were not significantly less effective than the best treatments (viz. those giving complete control) were used again in Trial 2 (Table 2). Exception was made for materials based on the same chemical, viz. parathion and schradan, trials being continued with only one of the compounds previously investigated. Figures in Table 1 are the means from 15 single-plant replications and those in Table 2 are the means from eight replications.

DISCUSSION OF RESULTS

Chlorinated hydrocarbons allowed a relatively large number of mites to survive treatment. Some organic phosphorus compounds gave contact effect alone, viz. mipafox (Isopestox), malathon (Malathion), and TEPP (Murphy Tepp); others gave both contact and residual

TABLE 1. TRIAL 1. EFFECT OF VARIOUS INSECTICIDES APPLIED AT
Mean of Three Replications, each of Five Plants

Chlorinated Hydrocarbons						Organic Phosphorus Compounds					
Treatment	lb./100 gal. water	Live mites per leaf after treatment				Treatment	lb./100 gal. water	Live mites per leaf after treatment			
		Mean No.		log (X + 1)				Mean No.		log (X + 1)	
		4 dys.	12 dys.	4 dys.	12 dys.			4 dys.	12 dys.	4 dys.	12 dys.
Check	—	4.1	30.3	0.72	1.50	Check	—	4.6	40.9	0.75	1.62
Aldrin em.	2	3.3	51.3	0.63	1.73	EPN-300	1.5	0.0	0.0	0.00	0.00
,,	1	3.4	27.5	0.65	1.48	,,	0.75	0.0	0.1	0.00	0.03
,,	0.5	2.3	5.0	0.52	0.75	,,	0.38	0.0	0.7	0.00	0.20
Aldrin w.p.	2	5.1	94.1	0.73	1.98	,,	0.19	0.6	5.5	0.19	0.79
,,	1	4.1	61.6	0.72	1.80	Folidol	1	0.0	0.0	0.00	0.00
,,	0.5	4.8	82.6	0.74	1.90	Fosferno 20	2	0.0	0.0	0.00	0.00
Chlordane em.	1	1.4	16.2	0.35	1.24	,,	1	0.0	0.1	0.00	0.02
,,	0.5	1.1	21.8	0.33	1.35	,,	0.5	0.0	1.9	0.00	0.47
,,	0.25	1.1	38.0	0.34	1.62	,,	0.25	0.0	5.2	0.00	0.79
Chlordane w.p.	1	1.9	20.0	0.48	1.32	Murphos 20	1	0.0	0.0	0.00	0.00
,,	0.5	1.5	23.1	0.39	1.39	Thiophos	2.5	0.0	0.1	0.00	0.04
,,	0.25	1.3	38.7	0.36	1.61	,,	1.25	0.0	1.6	0.00	0.40
Lindane 25	2	1.1	20.0	0.32	1.32	,,	0.63	0.0	35.0	0.00	1.56
,,	1	1.3	20.7	0.37	1.33	,,	0.32	0.0	31.1	0.00	1.51
,,	0.5	1.6	20.7	0.38	1.34	Metacide	1.3	1.2	4.8	0.34	0.76
						,,	0.65	0.6	6.4	0.19	0.87
						,,	0.32	1.0	4.8	0.31	0.77
						,,	0.16	0.3	3.0	0.10	0.61
						Hanane	1	0.0	0.0	0.00	0.00
						,,	0.5	0.0	0.0	0.00	0.00
						Isopestox	1.5	0.3	1.7	0.09	0.46
						,,	0.75	0.2	3.8	0.05	0.68
						,,	0.38	0.3	4.8	0.10	0.75
						Malathion	1	0.0	3.1	0.00	0.61
						,,	0.5	0.0	6.1	0.00	0.86
						,,	0.25	0.2	2.8	0.05	0.57
						Murphy Tepp	1.25	0.0	5.8	0.00	0.83
						,,	0.63	0.1	4.9	0.02	0.76
						,,	0.32	0.3	22.2	0.10	1.37
						Pestox 3	2	0.1	0.2	0.02	0.05
						,,	1	0.2	0.7	0.06	0.20
						,,	0.5	0.3	0.9	0.10	0.26
						Sytam	1.6	0.1	0.0	0.02	0.00
						,,	0.8	0.1	0.1	0.03	0.03
						,,	0.4	0.2	0.0	0.05	0.00
						Tetrax	0.8	0.0	0.0	0.00	0.00
						,,	0.4	0.0	0.0	0.00	0.00
						Systox	1	0.0	0.0	0.00	0.00
						,,	0.5	0.0	0.0	0.00	0.00
Difference for significance at:		5%	0.22	0.21						0.22	0.21
		1%	0.30	0.28						0.30	0.28

*Phytocidal effects. Treatments causing necrotic spotting on leaves the severity of which increased with higher concentrations.

SEVERAL CONCENTRATIONS ON THE RED SPIDER-MITE ON FRENCH BEANS

Miscellaneous Compounds						Miscellaneous Compounds (Contd.)					
Treatment	lb./100 gal. water	Live mites per leaf after treatment				Treatment	lb./100 gal. water	Live mites per leaf after treatment			
		Mean No.		log (X + 1)				Mean No.		log (X + 1)	
		4 dys.	12 dys.	4 dys.	12 dys.			4 dys.	12 dys.	4 dys.	12 dys.
Check	—	4.2	32.4	0.71	1.53	Check	—	5.0	43.6	0.79	1.65
Aramite	2	0.1	4.2	0.02	0.70	Marlate	5	1.7	15.3	0.42	1.21
,,	1	0.2	9.3	0.06	1.10	,,	2.5	2.4	6.1	0.49	0.87
,,	0.5	0.3	2.7	0.10	0.57	,,	1.25	2.4	9.1	0.50	1.00
Arathane*	2	0.8	7.0	0.25	0.90	,,	0.63	3.6	25.8	0.65	1.43
,, *	1	0.5	8.0	0.16	0.94	Murtox*	4	3.2	6.2	0.61	0.87
,, *	0.5	1.6	5.2	0.39	0.80	,,	2	2.7	10.2	0.56	1.05
Azobenzene w.p.	2.5	1.1	8.5	0.35	0.96	,,	1	2.1	16.2	0.49	1.24
,,	1.2	3.1	3.7	0.60	0.65	,,	0.5	3.6	19.5	0.67	1.32
,,	0.6	4.8	1.8	0.75	0.47	Nicotine Sulphate + soft soap	2.5	6.7	18.7	0.90	1.29
,,	0.3	6.3	8.8	0.85	0.99	,,	1.25	5.4	17.4	0.82	1.27
Mitosan A*	5	2.2	2.4	0.50	0.51	,,	0.63	5.1	6.6	0.78	0.88
,, *	2.5	1.6	3.0	0.35	0.60	Chlorocide	2.5	4.8	0.6	0.76	0.19
,,	1.25	3.7	9.4	0.68	1.00	,,	1.25	5.6	2.4	0.83	0.51
,,	0.63	5.5	16.0	0.81	1.23	,,	0.63	7.7	4.8	0.94	0.73
Mitosan B	2.5	3.7	12.8	0.66	1.14	,,	0.32	7.0	3.2	0.90	0.60
,,	1.25	5.6	10.7	0.89	1.07	Kloromite*	2.2	2.8	3.5	0.57	0.65
,,	0.63	7.2	14.9	0.91	1.19	,,	1.1	3.3	1.4	0.62	0.38
YF 2816	5	1.4	3.2	0.37	0.61	,,	0.55	2.3	1.1	0.50	0.32
,,	2.5	1.7	16.8	0.45	1.24	,,	0.28	4.3	1.1	0.71	0.34
,,	1.25	2.1	10.2	0.50	1.04	Murvesco	2	6.8	2.3	0.87	0.51
,,	0.63	5.1	12.5	0.80	1.13	,,	1	7.3	2.5	0.91	0.56
Colsul 40	8	2.9	9.5	0.58	1.00	,,	0.5	4.5	2.4	0.73	0.51
,,	4	2.5	6.2	0.54	0.86	Ovamite	2	6.9	2.4	0.90	0.53
,,	2	1.8	2.3	0.47	0.51	,,	1	4.3	2.7	0.72	0.58
DPS*	1	1.0	—	0.32	—	,,	0.5	3.7	1.3	0.66	0.39
,, *	0.5	1.1	3.0	0.32	0.63	,,	0.25	3.5	2.5	0.64	0.54
Dieldrin em.	2	2.6	12.6	0.54	1.31	Ovotran*	2	2.8	3.5	0.58	0.65
,,	1	1.9	5.5	0.48	0.87	,,	1	3.3	1.4	0.63	0.36
,,	0.5	3.2	10.5	0.61	1.06	,,	0.5	2.3	1.1	0.52	0.31
Dieldrin w.p.	2	1.8	35.3	0.47	1.58	,,	0.25	4.3	1.1	0.72	0.32
,,	1	1.3	21.8	0.35	1.35	PCPPCBS*	1	0.8	0.0	0.25	0.00
,,	0.5	2.1	21.6	0.48	1.34	,, *	0.5	2.1	0.0	0.50	0.00
Summer oil*	2†	0.7	0.0	0.20	0.00	Sulphenone*	2	0.3	0.5	0.10	0.16
,,	1†	1.1	1.8	0.30	0.43	,, *	1	0.9	2.2	0.29	0.50
,,	0.5†	1.9	30.6	0.46	1.51	,, *	0.5	2.1	4.1	0.48	0.69
Lime sulphur	1†	0.4	3.9	0.11	0.70	,,	0.25	1.7	11.9	0.40	1.09
,,	0.5†	1.7	14.2	0.46	1.19						
,,	0.25†	1.6	16.1	0.39	1.23						
				0.22	0.21					0.22	0.21
				0.30	0.28					0.30	0.28

† gallons,

TABLE 2. TRIAL 2. SELECTION OF THE MOST EFFECTIVE TREATMENTS IN CONTROL OF RED SPIDER-MITE ON FRENCH BEANS

Means From Two Replications, Each of Four Plants

Treatment	lb./100 gal. water	Live Mites per Leaf after Treatment			
		Mean No.		log (X + 1)	
		1 day	12 days	1 day	12 days
Treatments selected from Table 1					
Check	—	4.9	48.7	0.78	1.70
EPN-300	0.75	2.1	0.0	0.50	0.00
„	0.38	0.3	0.9	0.10	0.28
„	0.19	0.3	7.6	0.10	0.93
Fosferno 20	1	0.0	0.0	0.00	0.00
„	0.5	0.0	22.8	0.00	1.37
„	0.25	0.0	20.3	0.00	1.32
Metacide	0.65	0.2	0.3	0.05	0.10
„	0.32	0.0	14.6	0.00	1.19
„	0.16	0.2	14.7	0.06	1.18
Hanane	0.5	0.0	0.0	0.00	0.00
Isopestox	0.75	0.0	10.3	0.00	1.06
„	0.38	0.1	8.2	0.02	0.97
Murphy Tepp	0.63	0.0	89.0	0.00	1.95
„	0.32	0.0	60.6	0.00	1.79
Pestox 3	1	0.2	0.0	0.05	0.00
„	0.5	0.0	0.0	0.00	0.00
Malathion	0.5	0.0	54.3	0.00	1.74
„	0.25	0.0	41.7	0.00	1.62
Systox	0.5	0.0	0.0	0.00	0.00
Aramite	1	0.3	0.8	0.08	0.28
„	0.5	0.1	1.3	0.04	0.35
„	0.25	0.0	12.8	0.00	1.13
Summer oil	1.5†	0.0	0.0	0.00	0.00
„	1†	1.8	0.3	0.47	0.08
Lime sulphur	1†	3.4	42.2	0.65	1.64
Chlorocide	2.5	4.5	0.5	0.73	0.14
Difference required for significance at:		5%		0.26	0.27
		1%		0.34	0.31

† gallon

effects, viz. ethyl p-nitrophenyl benzene thiophosphonate (EPN-300), parathion (Folidol, Fosferno 20, Murphos 20, and Thiophos), dimefox (Hanane), schradan (Pestox 3, Sytam, and Tetrax), and demeton (Systox). Methyl parathion (Metacide) gave inconsistent results. Of the miscellaneous compounds, contact effect alone was brought about by butylphenoxyisopropyl chloroethyl sulphite (Aramite), residual effect

alone was shown by chlorparacide (Chlorocide), whilst petroleum oil (summer oil) was effective in both contact and residual capacities.

Although some substances such as chlordane, lindane, lime sulphur, and methoxychlor (Marlate) showed some contact effect, sufficient individuals remained to allow considerable build up of mite populations. Substances based on azobenzene (azobenzene w.p., YF 2816), p-chlorophenyl p-chlorobenzene sulphonate (Kloromite, Ovamite, Ovotran), and p-chlorophenyl benzene sulphonate (Murvesco) did not show contact effect and their residual effectiveness was inadequate under conditions of tests here described to reduce the level of mite populations to the same degree as that brought about by some of the organic phosphorus compounds. These compounds, however, did not allow populations to increase beyond the level ascertained at the first count and it is possible that a further reduction in number of mites might have been recorded had a third count been made.

CONCLUSIONS

Under these test conditions satisfactory control was obtained with the following materials, only the lowest effective concentrations being listed.

Contact action. EPN-300 at 0.019%, Isopestox at 0.038%, Murphy Tepp at 0.032%, Malathion at 0.025%, Aramite at 0.05% (Trials 1 and 2) and 0.025% (Trial 2), and parathion at 0.005% (Fosferno, Folidol, and Murphos 20 at 0.025%, and Thiophos at 0.032%). Metacide gave inconsistent results in both trials.

Residual effect. Chlorocide at 0.25%.

Contact and residual effect. EPN-300 at 0.038%, parathion at 0.02 to 0.037% (Folidol, Fosferno, and Murphos 20 at 0.1%, and Thiophos at 0.25%), Hanane at 0.05%, schradan at 0.028% (Pestox 3 at 0.05%, Sytam at 0.04% and Tetrax at 0.04%), Systox at 0.05% and summer oil at 0.8%. Metacide gave inconsistent results in both trials.

Insecticides of prolonged systemic action such as dimefox (Hanane), schradan (Pestox 3, Sytam, and Tetrax), and demeton (Systox), or others with a shorter systemic action such as mipafox (Isopestox) should not be used on other than ornamental plants until further information is available on their toxicity hazards.

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RELATIONSHIP BETWEEN RATE AND EFFICIENCY OF GAIN AND TYPE IN BREEDING BEEF CATTLE[1,2]

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(Received for publication, 17 August 1954)

Summary

The results of type classification and performance tests involving 42 purebred bulls and heifers of Hereford and Aberdeen Angus breeding have been analysed. These tests were conducted at Oregon State College, Corvallis. Factors studied were: Birth weight, weaning weight, rate of gain, efficiency of gain, type classification at 500 lb. and type classification at 800 lb. body weight.

(1) Type score increased with an increase in weight from 500 to 800 lb. body weight but the coefficient of repeatability was only 0.39.

(2) Neither type score at 500 lb. nor type score at 800 lb. body weight was significantly correlated with any of the production factors studied.

(3) A significant correlation existed between weight and rate of gain to 500 lb. Also a correlation existed between birth weight and rate of gain from 500 lb. to 800 lb. body weight.

(4) Post-weaning rate or efficiency of gain could not be accurately predicted from gains realized during the suckling period.

(5) Bull calves gained faster and more efficiently than heifer calves from birth to weaning and during the post-weaning test period (500 to 800 lb. body weight).

(6) Rate and efficiency of gain were highly correlated when measured through a weight constant period.

(7) Selection criteria for beef bulls and heifers cannot be considered adequate unless both type and production factors are included in a selection index.

INTRODUCTION

It is interesting to note that those who were largely responsible for the founding and early improvement of our present-day beef breeds used performance testing to determine the most efficient strains of cattle. Robert Bakewell conducted careful feeding trials as a means of production-testing his breeding herds, and others followed his example. According to Clark *et al.*[4], the show-ring came into being as a side light to performance testing of beef cattle. Early breeders, like their present-day descendants, frequently argued as to which had produced the fattest cattle and a disinterested third party was often chosen to act as judge. From such incidental beginnings the large stock shows of today have arisen. The testing of beef cattle for efficiency of production has been lost by the wayside only to be replaced by the belief that it is possible from certain observations of an animal to predict the future conformation, rate of gain, efficiency of gain, and transmitting ability of that animal. The advisability of selecting animals of a certain breed type with uniform colour markings and body type has been emphasized. Such beliefs in the ability of men to see in an animal the efficient producer without testing for production factors have been handed down from generation to

[1]This study was conducted in co-operation with Bureau of Animal Industry, U. S. Department of Agriculture, and State Experiment Stations under Western Regional Project W-1 on Beef Cattle Breeding Research. [2]Approved for publication as technical paper 882 by the Director of Oregon Agricultural Experiment Station. Contribution of the Department of Animal Husbandry.

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generation. It was the purpose of this paper to present the results of a study of this relationship to show whether or not such beliefs are founded on fact when breeding animals are involved.

Review of Literature

Animal husbandmen are beginning to realize the inaccuracy of the show-ring standard, and during the past 20 years experiments have been conducted to determine methods of appraisal of breeding animals based on progeny tests for economically important characters such as rate and efficiency of gain and carcass quality.

The literature on record-of-performance tests with beef cattle is relatively limited since such experiments with this class of livestock are expensive. Winters and McMahon[24] studied the results of an individually fed group of steers and found that there was a relatively high correlation between rate of gain and feed utilization. They suggested that an animal be evaluated on its weight at a year of age, its score or type, and fleshing. Sheets [20] proposed that a breeding animal be tested by the efficiency of meat production and carcass quality of his progeny. Black and Knapp[1] found that although there was a high correlation between rate and efficiency of gain, there was little or no correlation between rate or efficiency of gain before weaning and rate or efficiency of gain during the fattening period. These investigators, in studying the factors influencing gain during the suckling period, found that the quantity of milk, grain, and hay consumed accounted for a large proportion of the variation in gain during that period. Knapp *et al.*[10] further reported that daily gain and efficiency of gain are not highly correlated in a time-constant population and that score at weaning has a predictive value for final slaughter grade or carcass grade when animals are slaughtered at an average weight of approximately 900 lb., body weight. Winters[23] suggested that, in view of present knowledge of performance in beef cattle, testing should be based on: birth weight rate of gain during the suckling period; rate of gain or efficiency of gain during a standard weight period; and the animal's value for slaughter.

Knapp and Nordskog[13] determined the heritability of live-animal scores, grade, and certain carcass characteristics in beef cattle. Heritabilities were estimated by the use of intra-sire correlations and gave the following results: score at weaning, 53%; slaughter grade, 63%; carcass grade, 84%: and dressing percentages, 1%. These workers[14] also determined the heritability of growth rate and efficiency in beef cattle. They believed that the estimates of heritability derived in their study have been high, owing to the small size of population analysed, but that the influence of heredity on gaining ability and efficiency of feed utilization by calves may be high enough to make selection, based on individual performance for these traits, effective without the use of progeny testing. According to Lush[17], selection for a character within a small population of uniform animals does not necessarily give an indication of the general heritability of the character in the population as a whole. The heritability for weaning score in beef cattle was later lowered to 28%[12]. This is similar to the finding that type rating in Ayrshires is 28% heritable.

Hankins and Burk[8] reported a study employing feeder cattle from many experiments. They correlated several production and grade factors

in cattle and found that there was little or no relationship between feeder grade and subsequent gains of cattle in the feed lot. Patterson *et al.*[19] found practically no relationship ($r = -0.041$) between a type score at weaning and subsequent rate of gain in the feed lot. This was confirmed in a later study by Durham and Knox[6]. Knapp *et al.*[10] reported a correlation of 0.025 between score at weaning and subsequent gains in the feed lot with record of performance steers. Knapp and Clark[12] obtained a gross correlation of 0.0001 between score at weaning and gains in the feed lot. The heritability estimate for scores was 31%, and the gains 70%. It was concluded that there is little value for selecting feeder cattle for rapid gain if sole dependence is placed on a visual method of selection.

EXPERIMENTAL PROCEDURE

The data used in this study were from 42 beef calves maintained at Oregon State College, Corvallis, under the U.S. Western Regional Beef Cattle Improvement Project. The calves were pure-bred Hereford and Aberdeen Angus bulls and heifers. All were born during the spring of 1952 and were grazed with their dams on irrigated pasture until they were weaned at a body-weight of approximately 450 lb., or on 3 December, 1952. At this time they were placed under stall feeding conditions so that feed intake could be accurately measured. Individual stall feeding was done twice daily at uniform times. One-inch pellets composed of two parts half-ground lucerne and one part concentrate comprised the whole ration, which made it possible to determine feed consumption and refusal with accuracy[18]. All animals were fed so that there was some weighback daily, thus *ad libitum* feeding was simulated. All calves were weighed at birth and once weekly at a uniform time. Water was available at all times in drinking fountains.

Each calf was judged on conformation at both 500 and 800 lb. body weight by a committee of three qualified judges, each judging independently and using a score card to record results. Daily rate of gain from birth to 500 lb. body weight, birth to 800 lb., 500 to 800 lb., and efficiency of gain from 500 to 800 lb. were determined.

RESULTS

1. *Type Score*

Neither at 500 lb. nor at 800 lb. were the sex differences, or the sex × breed interaction, significant. In both cases, however, a significant breed difference was established, the type score for Herefords exceeding

TABLE 1. MEAN TYPE SCORES OF EXPERIMENTAL ANIMALS

Breed	No. of Animals	Body Weight		Both Weights
		500 lb.	800 lb.	
Hereford	26	81.5	83.7	82.6
Aberdeen Angus	16	78.2	79.5	78.9
Both Breeds	42	80.3	82.1	81.2
Difference		3.3	4.2	3.7

that for Angus. The analysis is shown in Table 3. The mean type scores are presented in Table 1.

Combining the data at both weights and ignoring the non-significant sex differences, the breed difference was found to be consistent, that is, there was no interaction (Table 4). Since the mean type score at 800 lb. was significantly greater than at 500 lb., it would appear that type increased with age and weight. As indicated in Table 1, the estimated increase was 1.8 units (%).

The significance of between-animal differences arranged over both weights indicates that it is possible to discriminate between animals (Table 4). However, the coefficient of repeatability (0.39) is hardly satisfactory in practice since only about 15% of the variation between animals at one weight, corrected for breed and time, is associated with a corresponding variation at the other weight.

2. *Birth Weight*

Both breed and sex differences were significant, but there was no evidence of significant interaction (Table 3). The estimated breed and sex differences, allowing for unequal numbers, were:

Breeds (Hereford—A. Angus) : 10.7 ± 2.2 lb.

Sex (Male—Female) : 5.1 ± 2.1 lb.

TABLE 2. AVERAGE BIRTH WEIGHT OF EXPERIMENTAL ANIMALS

	Male (lb.)	Female (lb.)	Both Sexes (lb.)	Difference (lb.)
Hereford	75.7	69.6	72.4	6.1
Aberdeen Angus	63.5	60.0	61.3	3.5
Both Breeds	71.6	65.6	68.2	
Difference	12.2	9.6		

TABLE 3. ANALYSIS OF VARIANCE: TYPE SCORES (AT 500 AND 800 LB.) AND BIRTH WEIGHT[1]

		Mean Squares		
Source of Variation	d.f.	Type Score 500 lb.	Type Score 800 lb.	Birth Weight
Breed	1	107.0**	166.5**	1,119**
Sex	1	1.0	16.4	265
Breed × Sex	1	3.1 (9.1)	0.2	15
Within Subclass	38	9.5	8.9	47

1. Allowance has been made in this analysis for unequal subclass numbers.

3. *Relationship between Type Score and Birth Weight*

The observed within-breed correlation coefficients were:

Type score at 500 lb. and birth weight: $r = 0$

Type score at 800 lb. and birth weight: $r = 0.28$ ($P < 0.1$)

There is little evidence therefore of significant association between the two measurements, type score, and birth weight.

4. *Relationships between Type Score and Rate and Efficiency of Gain*

The relationships between type score and the other production factors studied have been presented in Fig. 1. It may readily be seen

TABLE 4. JOINT ANALYSIS OF TYPE SCORE AT 500 LB. AND 800 LB.[1]

Source of Variation	d.f.	Mean Square	F.	Variance Component
Breed	1	278.3	22.23**	
Time (500 lb. or 800 lb.)	1	70.6	5.64*	
Breed × Time	1	4.5	0.36	
Calves (within breeds)	40	12.52	2.29	3.53
Residual (within calves)	40	5.46		5.46

[1]Sex difference ignored.

Repeatability $R = 3.53/(3.53 + 5.46) = 0.39$

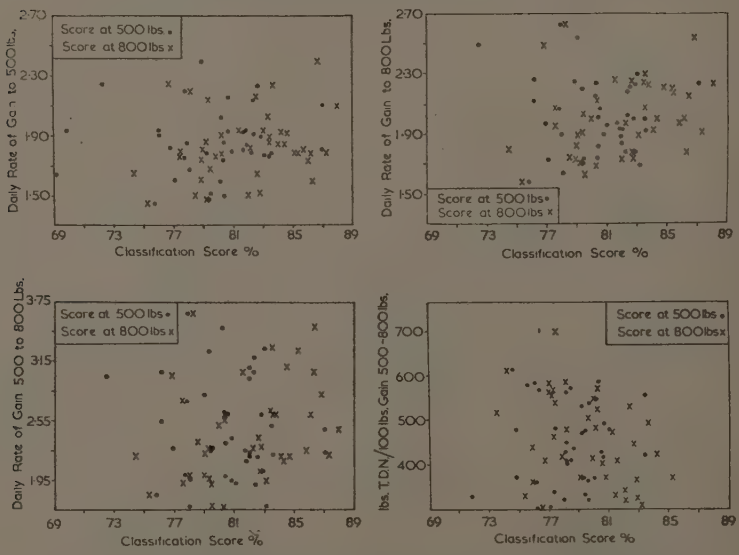
Score at 500 lbs.
Score at 800 lbs
Daily Rate of Gain to 500 lbs.
Daily Rate of Gain to 800 Lbs.
Daily Rate of Gain 500 to 800 lbs.
lbs. T.D.N./100 lbs. Gain 500-800 lbs.
Classification Score %

Fig. 1.—Relationship between type scores and production factors. *Top left*—Rate of gain: birth to 500 lb. *Top right*—Rate of gain: birth to 800 lb. *Bottom left*—Rate of gain: 500 lb. to 800 lb. *Bottom right*—Efficiency of gain: 500 lb. to 800 lb.

that the relationships between type score and rate of gain from birth to 500 lb., from birth to 800 lb., and 500 to 800 lb. were non-significant. Similarly the relationship between type score and efficiency of gain from 500 to 800 lb. body weight was also non-significant.

5. *Regression of Days from Birth to (a) 500 lb. (X_1), and (b) 800 lb. (X_2) on Birth Weight (Z).*

(a) *Days from birth to 500 lb. (X_1) on birth weight (Z)*: The within-subclass regression is significant only for Hereford females but the pooled regression (within breeds) is significant for both sexes. It was found that:

(i) The regression coefficients do not differ significantly between either breeds or sexes;

(ii) Allowing for the pooled regression, both breed and sex constants remained significant.

The appropriate regression equations and means are as follows:

		X_1	Z	
Male	Hereford	230.5	75.7	$X_1 = -2.31Z + 405$
	Angus	215.0	63.7	$X_1 = -2.31Z + 361$
Female	Hereford	247.8	69.6	$X_1 = -2.31Z + 408$
	Angus	252.1	60.0	$X_1 = -2.31Z + 390$

The pooled within-subclass correlation coefficient is $r(X_1Z) = -0.56$, and 31% of the within-subclass variation in X_1 is ascribable to variation in Z. The above regression suggests that each additional pound at birth weight represents a shortening by 2.3 days in the time taken to reach 500 lb. body weight (Table 5).

(b) *Days from birth to 800 lb. (X_2) on birth weight (Z)*: The analysis, which is not presented in full, follows the pattern presented in (a).

		X_2	Z	
Male	Hereford	335.2	75.7	$X_2 = -2.86Z + 552$
	Angus	321.2	63.7	$X_2 = -2.86Z + 503$
Female	Hereford	382.2	69.6	$X_2 = -2.86Z + 582$
	Angus	409.4	60.0	$X_2 = -2.86Z + 581$

$r(X_2Z) = -0.57$, and 32% of the variability in X_2 is ascribable to variation in Z (birth weight).

In the above equations two points are noteworthy. First, it is apparent that a significant association exists between birth weight and *rate* of *gain* from birth to both 500 and 800 lb. body weight. Secondly, the major discrepancy is the male Angus group, for which only a relatively few observations were available.

6. *Correlation between Days from Birth to 500 lb. (X_1) and from 500 lb. to 800 lb. (X) Body Weight*

In none of the separate subclasses, nor for the pooled data, is the correlation between X and X_1 found to be significant. Such a relation-

ship could, however, be marked by the negative correlation between birth weight and time to reach 500 lb. body weight. A multiple regression analysis was therefore carried out of X on X_1 and Z, equivalent to considering the regression of days from 500 to 800 lb. (X) on days from a constant birth weight to 500 lb. Neither the partial regression coefficients nor the multiple correlation coefficients were significant.

7. *Regression of Total Digestible Nutrients Intake (Y) on the Number of Days from 500 to 800 lb. (X) Body Weight*

Within each of the 4 breed-sex subclasses the regression of TDN on the number of days from 500 to 800 lb. (of Y on X) was significant (Table 5). It was also found that this relationship was the same for both breeds but a significant difference existed between sexes. Since there was no significance between breeds (in constant or Y intercept), the data may be summarized in the following equations:

Males: $Y = 4.69_x + 631.7 \pm 81.44 : (r_{xy} = 0.68)$

Females: $Y = 8.93_x + 291.1 \pm 97.26 : (r_{xy} = 0.87)$

TABLE 5. REGRESSION ANALYSIS: T.D.N. ON DAYS FROM 500 TO 800 POUNDS. AND DAYS FROM BIRTH TO 500 POUNDS ON BIRTH WEIGHT

		T.D.N. On Number of Days 500 to 800 Pounds			Days, Birth-500 lb. On Birth Weight	
		Deviations from Regression			Deviations from Regression	
		d.f.	b	m.s.	b	m.s.
Males	Within Hereford	10	3.87*		−1.94	
	Within Angus	4	6.19*		−2.23	
	Pooled within breed (separate regressions)	14		6,574		687.9
	Pooled within breed (common regressions)	15	4.62**	6,458	−2.06*	
	Difference between common and separate regressions	1		4,853		18
	Common regressions (ignoring breeds)	16	4.69**	6,632	−0.69	
	Difference due to breed intercept	1		9,228		3,892*
Females	Within Hereford	12	11.64**		−3.72**	
	Within Angus	8	7.35**		−1.13	
	Pooled within breed (separate regressions)	20		9,297		506.7
	Pooled within breed (common regressions)	21	8.37**	9,692	−2.53**	557.1
	Difference between common and separate regressions	1		17,600		1,564
	Common regressions (ignoring breeds)	22	8.93**	9,459	−1.77*	599.6
	Difference due to breed intercept	1		4,558		1,492

For males and females respectively, 43% and 75% of the variation in Y is accounted for by concomitant variation in X. In view of the high correlations found between Y and X, it is evident that there is also a strong association between rate of gain and efficiency of gain in the weight-constant period from 500 to 800 lb. body weight. The respective breed and sex means are presented in Table 6.

TABLE 6. RATE OF GAIN, AVERAGE NUMBER OF DAYS AND POUNDS OF TOTAL DIGESTIBLE NUTRIENTS REQUIRED FOR GAINS FROM 500 TO 800 POUNDS BODY WEIGHT

			Hereford	Aberdeen Angus	Both Breeds
MALES	Efficiency of Gain	Total T.D.N.*	1107	1162	1125
		T.D.N./lb. Gain	3.69	3.87	3.75
	Rate of Gain	Days	104.7	106.2	105.2
		Gain/Day (lb.)	2.87	2.82	2.85
FEMALES	Efficiency of Gain	Total T.D.N.	1490	1716	1584
		T.D.N./lb. Gain	4.97	5.72	5.28
	Rate of Gain	Days	134.4	157.3	144.0
		Gain/Day (lb.)	2.23	1.91	2.08
BOTH SEXES	Efficiency of Gain	Total T.D.N.	1313	1508	1388
		T.D.N./lb. Gain	4.38	5.03	4.63
	Rate of Gain	Days	120.8	138.1	127.3
		Gain/Day (lb.)	2.48	2.17	2.36

*T.D.N. = TOTAL DIGESTIBLE NUTRIENTS = digestible carbohydrates + digestible protein + (digestible fat × 2.25).

Discussion

In the selection of breeding animals it is important to determine their potential value at the earliest possible age. This is particularly true in beef cattle since most males are castrated at or before weaning because of the preferential price return enjoyed by the castrated animal. The selection of females at an early age is neither as critical nor as intense. It would be preferred, therefore, to make both type and production factor selection at or prior to weaning. It appears from the results of this experiment that selections at weaning (500 lb.) for type at a later period of physiological development are somewhat hazardous, but compensated for by a general rise in type classification with growth and the presence of many steers of good if not excellent conformation. Not only is the selection for breeding animals based on conformation at 500 or 800 lb. far from foolproof, but also it is not highly correlated with factors of commercially economic value such as rate and efficiency of

gain. Production factors, at present, are of considerably greater value to the commercial producer than carcass conformation which is dependent to such a high degree on plane of nutrition, an environmental, not a genetic, factor. The results of this study agree with the findings of Patterson *et al.*[19] and Durham and Knox[6] that practically no relationship exists between a type score at weaning and subsequent gain in the feed lot. The report by Koger and Knox[15] that the growth rate of steers on pasture was positively correlated with their gains in the feed lot would indicate that such findings apply equally well to cattle finished entirely on pasture. Therefore, since many generations of selection based entirely on conformation has not resulted in animals in which type and production factors are highly correlated, one must assume that the belief that a show-ring selection for production factors is possible, is not valid.

Although size, as such, and conformation are not fundamentally related to efficiency, [2 3 21 22] selections based on "weight for age" should be considered a criterion of desirability in meat animals. Energetic efficiency comparisons can only be considered valid when the energy content of the growth increments, as well as the energetic intake, are equal. However, since slaughter animals are not bought calorically but largely on the basis of weight, and to a lesser degree finish (fat), and since the caloric content of the breeding animal is not readily determinable, efficiency of gain will undoubtedly be assessed for some time on the basis of simple gross weight gained through comparable segments of growth based on weight. The assessment of efficiency of gain is further complicated by the difficulties involved in assessing the qualitative and quantitative feed consumption of grazing animals. Because rate and efficiency of gain in a weight-constant period are positively correlated [1 10], selection for rate of gain should be adequate [16] until such times when far more intensive selection demands a more accurate assessment of efficiency itself.

While neither type score at 500 lb. nor type score at 800 lb. was significantly correlated with birth weight, the significant correlation existing between birth weight and rate of gain, both to 500 lb. and from 500 to 800 lb. body weight, justifies the recommendation[5 23] that birth weight be included among the component factors comprising a selection index for beef cattle.

That production testing for factors involving rate and efficiency of gain is a necessary but heretofore neglected complement to the selection of breeding through conformation criteria is obvious from the data reported. A completely satisfactory method of testing for such factors under grazing conditions has yet to be presented.

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THE EFFECTS AND INTERACTIONS OF SULPHUR, PHOSPHORUS, AND MOLYBDENUM ON THE GROWTH AND COMPOSITION OF CLOVERS

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Summary

Results are reported of trials carried out to assess the effects of sulphur, phosphorus, and molybdenum on the yield and composition of oversown clovers in the Canterbury foothills. On the average, sulphur was more important than phosphorus, but both were needed to get good establishment and clover growth. Molybdenum had no significant effect on growth in the first year but increased the Mo content of the herbage markedly in some cases. "Available" soil phosphate correlated well with responses to P but not to P + S. Gypsum depressed the yield of clovers on one soil and this is attributed to an increase in Mn uptake and intensified Mn–Mo antagonism. The significance of sulphur in New Zealand pastoral farming is discussed and it is suggested that the greatest need for sulphur may arise during the phase of organic-matter accumulation under pastures. This is dependent primarily on N fixation by clovers and necessitates an increase in C, organic S, and organic P, if the C:N:organic S:organic P ratio in soils is assumed to remain fairly constant.

Introduction

One of the primary causes of poor pasture growth is low production from legumes, with consequent dependence of the grasses on mineralized soil nitrogen as their main source of N. A surprising feature of many of the natural grasslands of the world is the relative absence of legumes and in this respect the tussock grasslands of New Zealand are no exception. The improvement of the unploughable tussock grasslands depends on over-sowing with suitable legumes (and perhaps eventually grasses) and the application of the necessary nutrients. The aeroplane has greatly facilitated these operations and it remains among other things to decide exactly which legumes are most suitable, the best methods of introduction and of management before and after introduction, and the diagnosis and correction of nutrient deficiencies. This paper is concerned primarily with the last factor on some representative Canterbury foothill soils.

Recent work in New Zealand indicates that on many soils perhaps the most important nutrient deficiencies as far as legumes are concerned may be phosphorus, molybdenum and/or lime,[1 2 3] sulphur,[4 5 6 7] and potassium[8]. The only nutrients investigated in these trials were S, P, Mo, and lime.

Experimental

Treatments and Lay-out

Six trials were put down in October–November 1953, ranging from Lake Coleridge in mid Canterbury to Cheviot in the north. The trials were 2^3 factorials for S, P, and Mo, randomized in blocks with 2 or 4 replicates. Lime was superimposed non-factorially on half the blocks. Rates per acre were as follows:

S = $CaSO_4$ at 100 lb.,
P = Monocalcium phosphate (triple super) at 100 lb.,
Mo = Sodium molybdate at 4 oz.,
Lime at 250 lb.

The SP treatments approximate to a dressing of superphosphate of about 2 cwt. per acre. The low rate of lime was decided on to keep the total amount of added materials to a level it was thought practicable to apply from the air; a higher rate may have been better. When putting down the first three trials it was found almost impossible to find a uniform site because of slopes, rocks, and the uneven distribution of tussock grasses and weed shrubs such as matagouri. For the last three trials the plot size was increased from 5 to 10 sq. yd. and the replicates reduced from 4 to 2. The trial areas were fenced and netted, treatments applied by hand and the whole area oversown with the following clover mixture:

	lb. per acre
N.Z. certified white clover (inoculated)	6
Subterranean clover	6
Montgomery red clover	6
Alsike	6
Total	24

These seed rates may appear heavy, but the main objective was to get clovers established and to see which species thrived. A mixture of grasses (cocksfoot, ryegrass, dogstail, Yorkshire fog and *Phalaris tuberosa*) was also sown, but at the time of cutting in April 1954 (after 5 months) very few of the sown grasses were evident and no attempt was made to assess their establishment.

At harvest, the clovers were scored visually for establishment and growth (giving 10 marks to the best plot and marking the others accordingly), and where clovers had made sufficient growth a square-yard cut was taken from each plot. Clovers were separated from grasses and weeds, and the two components weighed and dried for subsequent analysis.

Soils

Windwhistle. This trial was situated on a terrace above the Rakaia river on an old sown pasture sparsely populated with stunted clovers which had not been topdressed for 11 years. Rainfall for the area is 44 in., well distributed, and the altitude is about 1,400 ft. The soil type is Kowai fine sandy loam, a young loessial soil with 10–12 in. of light-grey topsoil of weak structure on light-yellowish-brown fine sandy subsoil. An unusual feature of this soil for Canterbury is the high content of available phosphorus.

Oxford. The trial at Oxford, on a well grassed area containing some rushes, was situated at about 1,300 ft. Rainfall for the area is about 45 in. The soil type is Oxford silt loam, a very strongly leached yellow-grey earth. Topsoil is a very-pale-grey silt loam on a pale-yellow heavy silt loam to clay loam subsoil with reddish-yellow mottling. A possible explanation for the high figure for exchangeable calcium may be that the bush on the area has been burned in quite recent times.

Lake Coleridge. The plot at this site was on a badly wind-eroded flat, subject to severe frost lift and high winds, and at an altitude of about 1,800 ft. Rainfall is 35–40 in. The soil, with 9 in. of brown weak-structured topsoil is classified as Cass fine sandy loam, a strongly leached, weakly weathered yellow-brown earth, typical of the intermontane basins in the Canterbury high country.

Lees Valley. The trial area, at 1,500 ft. and with a rainfall of 45 in., was situated on a hill soil carrying unimproved tussock, soil type being a gleyed associate of Hurunui silt loam, with 8 in. of very-dark-grey stony silt loam topsoil of weak structure on pale-yellow silt loam subsoil with reddish-brown mottling and concretions.

Haydon Downs. Situated on a small alluvial flat of unimproved tussock, this plot is on a soil type associated with the Hurunui series, as yet undifferentiated, which occurs on small terraces throughout the high country. The topsoil of 9 in. is a dark-brown stony silt loam. Rainfall is 45 in. and altitude 1,400 ft.

Domett. Unimproved tussock on hill country on the north bank of the Hurunui River; rainfall 35 in., altitude 300–400 ft. This plot is on Cheviot silt loam, a moderately leached, weakly gleyed, yellow-grey earth with 9–10 in. of very-dark-grey fine sandy silt loam of weak structure, derived from slightly calcareous Tertiary silts, overlying a mottled heavy silt loam.

The A horizons of these soils were analysed and the results are shown in Table 1.

TABLE 1. RESULTS OF SOIL ANALYSES*

Centre	Wind-whistle	Oxford (Clay Downs)	Lake Coleridge	Lees Valley	Haydon Downs	Domett
Soil Series	Kowai	Oxford	Cass	Hurunui	—	Cheviot
pH	5.3	5.3	5.3	5.3	5.5	5.7
T.E.C.(m.e.%)	12.5	26.4	16.4	18.1	22.0	15.5
Exch.Ca(m.e.%)	4.9	11.5	2.3	4.6	7.1	3.8
W/sol. + Exch.Mn(p.p.m.)	2.8	7.1	1.4	12.8	5.6	20.3
Easily reducible Mn (p.p.m.)	62	172	19	29	56	67
% C	3.7	6.0	3.7	4.0	6.1	3.4
% N	0.28	0.42	0.25	0.25	0.45	0.25
% S	0.034	0.043	0.019	0.025	0.047	0.031
Avail.Mo(p.p.m.)	0.74	0.11	0.17	0.11	0.03	0.21
Truog P_2O_5(mg.%)	9.0	4.8	1.4	4.5	4.1	2.5
N/S ratio	8.2	9.8	13.2	10.0	9.6	8.1
C/S ratio	109	140	195	160	130	110
C/N ratio	13.2	14.3	14.8	16.0	13.6	13.6

*Analytical methods for soils and plants were those listed by us elsewhere[2,7].

RESULTS

Scores and Yields of Clovers

Growth of clovers was outstanding in most trials on plots where the treatments happened to supply the missing nutrient or nutrients. White and subterranean clovers were normally dominant with a few plants of red clover and alsike. On other plots clovers were conspicuous by their absence or dwarfed appearance. In some cases the clovers in the absence of sulphur were markedly yellow—a rather brighter yellow than is normally associated with straight N-deficiency. Both the scores and yields (where obtained) were treated statistically and the results are presented in Table 2.

TABLE 2. MEAN SCORES (MAX. 10) AND YIELDS OF CLOVER,† (D.M. lb./ac.)

Treatment	Windwhistle	Oxford (Clay Downs)	Lake* Coleridge	Lees** Valley	Haydon Downs	Domett	Relative Mean Scores (omitting Domett)
O	1 (67)	2 (9)	1½	½ (Trace)	0 (0)	3½ (41)	12
Mo	1 (47)	1½ (14)	0	0 (Trace)	0 (0)	4 (52)	6
P	2 (103)	4½ (86)	4½	3½ (10)	1½ (22)	8 (290)	38
S	8 (866)	4½ (64)	2	1½ (Trace)	5 (175)	1½ (19)	49
PMo	1 (70)	4 (69)	4½	4½ (33)	1½ (13)	7 (288)	36
SMo	8 (792)	5 (96)	3½	1½ (Trace)	4½ (155)	1½ (10)	53
SP	8½ (986)	9 (406)	9	6 (73)	8½ (465)	5½ (70)	96
SPMo	9 (1088)	9 (531)	8	8½ (196)	8 (497)	6 (268)	100
Sig. diff. at 5%:	1.5 (316)	2.5 (358)	1.3	3.2 (−)	1.7 (167)	1.8 (196)	

† in brackets.
* Clover not sufficiently grown to cut.
** Separate plots pooled for weighing and yields are only approximate.

It is worth commenting on the fact that the scores indicate more significant differences than the yields. Both are objective measures as the scores were made without reference to the randomization plans. The reason for the differences is unquestionably the uneven distribution of the tussock grasses; allowance was made for this during scoring (the establishment and growth of clovers around the tussocks guiding the scorer), but when cutting a square yard for yields, the presence or absence of tussocks had a big effect.

Omitting Domett which will be considered separately, the relative mean scores show that, on the average, S alone has been slightly more effective than P alone, but apart from Windwhistle and Domett there was a marked positive SP interaction, shown rather better by the yield data than the scores, indicating deficiencies of both S and P. Mo had surprisingly little effect even in the presence of S + P, but this may be due to the fact noted by Anderson and Moye[9] that nodulation of clovers may be slow on acid soils, and molybdenum gives a delayed effect in such cases. Anderson and Moye[9] have shown that as little as two

cwt. $CaCO_3$ per acre drilled with the seed encourages rapid nodulation and immediate response to Mo, but this effect was not noted in these trials, possibly because the topdressing of 250 lb. $CaCO_3$ was much less effective than placement. The lime treatments, with so few replications, were only significantly better than no lime at Lake Coleridge and will not be considered further.

The insignificant response to P or PMo compared with the S response at Windwhistle is striking, but confirms the Truog phosphate level which was satisfactory. The only significant responses to P alone or PMo were at Coleridge and Domett, which on analysis were the two soils most deficient in P. Definite, but statistically insignificant, responses were obtained to P at Oxford, Lees Valley, and Haydon Downs, where the Truog phosphate levels were medium. S alone or SMo gave significant responses at Windwhistle and Haydon Downs, but slight responses were obtained at all other centres except Domett where the yield was depressed (significantly according to the scores). At Domett, SP gave a significantly lower yield than P or PMo, and was little better than the control, whereas SPMo gave a yield similar to P or PMo. As will be shown later, this depression from S is probably due to the effect of gypsum increasing Mn-uptake; this interferes with the role of molybdenum in N fixation and reduces the yield[3]. Correction of this Mn–Mo antagonism by the application of SPMo restores the situation.

In the two extremes of these trials, we have notable examples of the possible misinterpretation of the value of different phosphate fertilizers. Superphosphate would have given a marked response at Windwhistle on a P-rich soil due to S deficiency, and would have given no response on the P-deficient soil at Domett because the gypsum it contains intensifies Mn–Mo antagonism and gives rise to a condition where molybdenized superphosphate (SPMo) may be superior to superphosphate but not to monocalcium phosphate.

There have been more attempts to correlate crop responses to phosphate fertilizers with soil analytical data than for any other nutrient, and many attempts made to account for anomalous result (e.g. Cooke[10]). Superphosphate is the commonest fertilizer used in this type of experiment, and it is obvious that cases of S or Mo deficiencies, or induced Mn toxicity may account for some of the discrepancies. Similarly, superphosphate is usually taken as the standard when carrying out experiments to evaluate other forms of phosphate fertilizers; its superiority or inferiority on certain soils may often be due to the gypsum it contains.

If our trials had been conducted in an attempt to correlate soil P data with responses to superphosphate it might have been concluded that the particular method of soil analysis used was useless on such soils; as it happens the soil analyses would have been more reliable than the misinterpreted field trials (Fig. 1). Whilst there are too few data to be dogmatic, there is obviously a much closer correlation between soil analysis and P responses than with SP responses. (Scores were used to calculate $\frac{1}{2}$(P + PMo − O − Mo) and $\frac{1}{2}$(SP + SPMo − O − Mo).

The responses to S (as measured by the scores) were similar in the presence or absence of P, and do not correlate at all with the percentage of S in the soil, indeed the three soils with the highest percentage of S gave the biggest yield responses. It would have been surprising if a

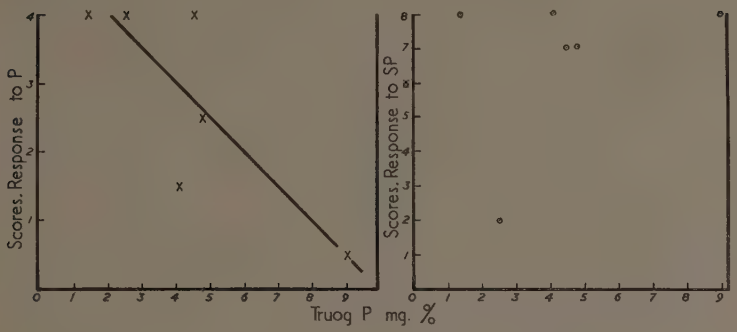

Fig. 1.—Responses to P and to SP plotted against Truog P.

good correlation had been obtained, because if we can compare nitrogen with sulphur it is well known that correlations between percentage of N in soils and yields of dry matter are often poor, although there may often be good correlation with the yields of N in non-legumes and percentage of soil N[11]. Pushing the comparison further, it is frequently observed that the amount of mineral N produced on incubation correlates with percentage of soil N, and a good correlation might be expected between total soil S and S mineralized on incubation. This is a possibility we are examining at present. In this respect high C:S ratios in soils may tend to produce S deficiency, but the most S-responsive soil has the lowest C:S ratio (Windwhistle). The problem is extremely complicated in associations of grasses and legumes because of competition for nutrients; with a soil fairly rich in N and poor in S, grasses will be stimulated and may take up most of the available sulphate, leaving little if any for legumes; with a lower soil N and poorer grass growth, there may be more S available for legumes. This aspect will be discussed further below, but it is of interest that the three most S-responsive soils contain the highest percentage of N, although, as shown in Table 1, there is a fairly constant N:S ratio.

Composition of Clovers and Grasses

Owing to the absence or very poor growth of clovers with some treatments, the analytical data are unavoidably fragmentary, and are mainly useful in illustrating trends.

Nitrogen. These data are presented in Table 3.

In all cases SP has clearly increased the percentage of N in the clovers compared with P, and SPMo has given further increases, confirming the data of Anderson and Spencer[12]. Although Mo had little effect on yields, it consistently increased the percentage of N in the clovers. The grasses contained very little nitrogen, but were rather mature; nevertheless the data confirm those of Coop *et al.*[13]. The yields of grasses varied from about 2,000 to 3,500 lb. dry matter per acre, giving low yields of N, and nitrogen is clearly one of the main factors limiting production. Even on the highest-yielding plots, the yields of clover N were very small when compared with the large amounts of N fixed under the best New Zealand conditions, but since these yields represented only 5 months' growth from seed, higher yields could be expected in the

TABLE 3. PERCENTAGE N IN DRY MATTER AND YIELDS OF N* (lb./ac)

Centre	Clovers			Grasses
	P	SP	SPMo	Mean of all analyses and yields
Windwhistle	2.13(2)	2.42(24)	2.82(31)	0.98(34)
Oxford	2.48(2)	2.95(12)	3.10(16)	1.33(41)
Lees Valley	2.16(0.2)	2.34(2)	2.89(6)	— —
Haydon Downs	2.31(0.5)	3.09(14)	2.91(15)	1.17(22?)
Domett	2.59(8)	2.70(2)	3.09(8)	0.96(22)

* in brackets

following years. Even so, at Windwhistle, clovers yielded almost as much N as the grasses, and if grazed would have added materially to the amount of N in circulation.

Phosphorus. As shown in Table 4, application of P usually gave a marked increase in percentage of P in the clovers, which was very low in the S treatments. The application of P alone had only a small effect

TABLE 4. PERCENTAGE P IN DRY MATTER

Centre	Clovers				Grasses
	S	P	SP	SPMo	S
Windwistle	0.11	0.28	0.27	0.17	0.23
Oxford	0.13	0.26	0.21	0.17	0.07
Lees Valley	—	0.34	0.15	0.15	0.06
Haydon Downs	0.12	0.24	0.20	0.15	0.10
Domett	0.12	0.11	0.17	0.15	0.11

on yield (except at Domett) and an increase in percentage of P might therefore be expected. Where SP was applied, the percentage of P tended to fall, perhaps because of the relatively large increase in dry matter (except at Domett where SP reduced the yield compared with P and here the percentage of P increased). SPMo tended to reduce the percentage of P slightly compared with SP. The P content of the S-treated grasses was very low apart from Windwhistle where the soil was richest in available P.

Sulphur. Both total and inorganic S were determined in clovers and grasses from some treatments, as shown in Table 5. The yields of clovers in the controls were so poor that the P-treated plots have had to be taken as the standard to compare with treatments receiving S.

The percentage of total S in the P-treated clovers is well below the "critical limit" of 0.2% S given by Goodall and Gregory[1] for lucerne at all centres, but above the levels (0.05 to 0.06) given by Anderson

TABLE 5. PERCENTAGE TOTAL S AND INORGANIC S* IN DRY MATTER

	Clovers				Grasses	
Centre	P	S	SP	SPMo	O	S
Windwhistle	0.078 (0.013)	0.147 (0.010)	0.109 (0.022)	0.151 (0.014)	0.061 (0.016)	0.115 (0.058)
Oxford	0.167 (0.043)	0.188 (0.060)	0.197 (0.051)	0.233 (0.041)	0.117 (0.007)	0.216 (0.045)
Lees Valley	0.104 (0.060)	— (—)	0.150 (0.061)	0.187 (0.032)	— (—)	— (—)
Haydon Downs	0.107 (0.056)	0.155 (0.034)	0.162 (0.009)	0.169 (0.055)	— (—)	0.188 (—)
Domett	0.134 (0.006)	0.187 (0.026)	0.171 (0.027)	0.172 (0.023)	0.132 (0.053)	0.176 (0.081)

* in brackets

and Spencer[12] for subterranean clover grown on S-deficient soils. All treatments containing S increased the percentage of total S markedly, in both grasses and clovers. At the centre most responsive to S (Windwhistle) clovers and grasses contained the lowest percentage of total S. This centre also gave the lowest figures for percentage of inorganic S. Where S deficiency is seriously limiting N fixation by clovers, inorganic S might be expected to be low, and it is interesting to note that the percentage of inorganic S remains fairly constant in the clovers, suggesting that the additional S taken up on the S-treated plots is all converted to protein or to S-containing amino-acids. In marked contrast, almost all the extra S taken up by the grasses remains in the inorganic form, presumably because N was a more limiting factor in their growth, thus confirming the data of Wood and Barrien[15]. It is of interest that SPMo usually caused an additional increase in organic S compared with SP in the clovers, as it did for total N; the total-N:organic-S ratios usually fell markedly (e.g. Windwhistle 32, 28, 21 for the P, SP, and SPMo treatments respectively). This ratio is about 20 at all centres for all the SPMo treatments and is therefore rather higher than the fairly constant ratio of protein-N:S of 12 to 14, found by Wood and Barrien[15] for grasses.

Our data suggest that there may be luxury consumption of SO_4 by grasses, as a much bigger fraction of the S taken up is in the inorganic form compared with clovers, and grasses may compete rather effectively with clovers for SO_4. Work by Askew and Bishop[16] supports this statement, where, even at a very high level of S nutrition, grasses contained more S than clovers, with a much higher proportion in the inorganic form. For instance at Windwhistle, on the control plots the grasses and clovers took up about 2 lb. and 0.1 lb. S per acre respectively, whereas on the S-treated plots the uptakes were 4 and 1.5 lb. S; without any apparent increase in yield, the grasses took up more of the added S than the clovers despite the extra 1,000 lb. dry matter of clover produced per acre.

Molybdenum. The data for the SP and SPMo treatments are given in Table 6. Although there was little difference in yields of dry matter of clovers for these treatments (except at Domett), molybdenum has effected a pronounced increase in the molybdenum content of the clovers.

TABLE 6. Mo IN DRY MATTER (p.p.m.)

	Clovers		Grasses	
Centre	SP	SPMo	SP	SPMo
Windwhistle	0.9	4.1	2.0	4.4
Oxford	4.6	23.5	1.8	8.7
Lees Valley	1.0	9.2	1.3	4.0
Haydon Downs	1.4	7.5	2.4	4.7
Domett	0.4	0.9	1.8	2.2

The molybdenum content of the SP-treated clovers does not correlate at all with the "available" molybdenum[17], and, as the pH values for the soils are similar, allowances made for varying pH as suggested by Davies[18] do not improve the correlation. Further, the soils which would be considered deficient on the basis of soil analysis have not given yield responses to Mo. This may however be due to a delayed effect as previously noted[3]. The Mo contents do, however, correlate very well with exchangeable calcium which is highest at Oxford and lowest at Domett. The question of Mn toxicity and Mn–Mo antagonism will be discussed below, as these are certainly other factors to be considered.

Of particular interest is the increase in Mo content on application of Mo, from the startling effect at Oxford to the slight effect at Domett. These increased concentrations might have been less if Mo had effected any marked increase in yields of dry matter, as it did at Domett. The data at least underline the warning pronounced by Cunningham[19] on the risks to stock health attending indiscriminate use of Mo. The Mo contents of the SP treatments are all higher than the critical level of 0.1 p.p.m. Mo suggested by us[3] for deficiency cases uncomplicated by high uptake of Mn.

In the case of grasses, in the absence of Mo the molybdenum contents were often slightly higher than in the clovers, but did not normally increase as much with the application of Mo.

Manganese. In view of the effects of Mn toxicity and the definite evidence of Mn–Mo antagonism[3] on the growth of clovers, it is of some interest to examine the Mn contents of the clovers and grasses, and these data are presented in Table 7.

It has been suggested elsewhere[3] that when the Mn content of white clovers exceeds 60 to 80 p.p.m. it may affect the role of Mo in N fixation, but that the application of more molybdenum may further stimulate N fixation and production of dry matter and effect a reduction in the Mn content. E. G. Mulder (priv. comm.) suggests that, in the case of cauliflower, Mn may even reduce uptake of Mo.

TABLE 7. Mn IN DRY MATTER (p.p.m.)

	Clovers					Grasses	
Centre	O	S	P	SP	SPMo	O	S
Windwhistle	165	112	117	117	111	241	220
Oxford	145	128	102	112	113	364	363
Lees Valley	—	—	245	202	212	124(SP)	177(SPMo)
Haydon Downs	—	155	167	146	167	—	362
Domett	204	275	254	281	258	607	745

The control clovers (or P-treated clovers where there were no control clovers) all contained more than 100 p.p.m. Mn, and Mn toxicity or Mn–Mo antagonism may be responsible as well as S deficiency for the poor growth of clovers on the control plots. At Windwhistle where, according to sulphur responses and clover analyses, S deficiency was quite acute, the application of gypsum lowered the Mn content from 165 to 112 p.p.m., but it is to be noted that exchangeable Mn is comparatively low in this soil and that the total uptake of Mn is much higher on the S-treated plots. At Domett, on the other hand, where certainly from the clover analyses S deficiency cannot be discounted, application of gypsum reduced the yield. It is notable that this soil contained most exchangeable Mn, and gypsum has been observed to increase Mn uptake on acid soils[20] [21]. At this centre also, where available Mo in the soil was quite high but where clovers contained least Mo, it is possible that a toxicity level of manganese is an important factor in preventing possible S responses and in reducing the uptake of Mo. Indeed on acid soils, high in exchangeable Mn, it is difficult to see how S deficiency can be corrected without initial liming.

The rather good correlation between exchangeable Mn in the soils and Mn content of the clovers is worth noting, as is the rather high Mn content of the grasses at Domett. It is difficult to see where easily reducible Mn fits into the picture, but this may not be a very significant factor in cases of Mn toxicity.

Calcium. This was determined only for the SPMo treatments on clovers and was 1.18%, 1.45%, 1.51%, 2.14%, and 2.36% for Domett, Windwhistle, Oxford, Haydon Downs, and Lees Valley respectively. The exceptionally low figure for Domett is noteworthy, lending emphasis to the singular effect of the acidity complex at this centre. The figures again confirm other analyses in the high country[13].

DISCUSSION

The significance of S in New Zealand in relation to pastures and top-dressing is worth looking at in some of its wider aspects. It is of some importance to assess the general need for sulphur as a nutrient, but total soil S does not seem to be correlated at all with S responses. Unquestionably most of the sulphur in soils is associated with the organic matter, and, arguing from the knowledge of soil organic N and its mineralization, a high C:S ratio may be an important factor. Whilst

this ratio appears to be high for some of our trial soils, clovers, lucerne, and rape responded to S in North Otago[5] on soils with much lower C:S ratios of about 80[22]. A general observation is that initial responses to S on pastures are given by clovers[23], but subsequently, owing to the increased N status, grasses may also respond. The inference is that grasses may utilize most of the mineralized N from soil organic matter (we have as-yet-unpublished support for this suggestion), and they may also compete strongly for mineralized S, so that unless organic S is mineralized at a relatively greater rate than organic N, only small amounts of sulphate may be available for clovers. Clearly, in grass–clover associations it is not possible to isolate the sulphur cycle and discuss it without reference to the C and N cycles and possibly the P cycle, although P is different from C, N, and S in that a fairly large fraction of the total P in soils may exist in the inorganic form.

Evidence is accumulating[24 25] that soil organic matter contains C, N, and P in fairly constant proportions, which for the purposes of discussion may be taken at 100:10:1. Most of the available data suggest that organic S could be included in this picture with C:N:P:S being approximately 100:10:1:1. Until recently our views on the rate of accumulation of C and N in pasture soils have been greatly influenced by Richardson's early work at Rothamsted[26]. Sears *et al.*[27], and Metson and Hurst[28], have recently published data indicating that under New Zealand conditions there is a much higher rate of accumulation of C and N. Indeed a theoretical treatment of the Palmerston North data[11], based on Jenny's mathematical approach to the accumulation of C and N[29], shows that in some of the best pasture soils of New Zealand, where N may accumulate at the rate of 400 to 500 lb. per acre per annum (owing to high N fixation by clovers), the eventual equilibrium level of soil N may easily exceed 1%, after a period of about 50 years. This very large increase in N must, presumably, be accompanied by an increase in C, organic P, and S, and, if the above proportions still hold at these high levels, an accumulation of 500 lb. N in the soil would entail a corresponding addition of 50 lb. organic P and S, or approximately the P and S contained in 5 cwt. superphosphate. Normally, less than this amount would suffice as some S would be added from the air, and soil inorganic P may act as a further source for the formation of organic P, gradually leading to a condition where organic P forms by far the biggest fraction of the total soil P[30 31]. If S and P are not added to the soil in amounts sufficient to meet demands, clover growth and N fixation may suffer first, with perhaps subsequent widening of the C:N, C:S, and C:organic P ratios.

The significant fact is that the need for S (and P) follows from the large amounts of N and C that may be built up under New Zealand pastures, and is part of the price to be paid for the accumulation of organic matter under pastures. It would be of some considerable interest to compare the rates of mineralization of organic N, S, and P under various environmental conditions, although, as recent work shows in the case of nitrogen[32], there is a continuous cycle of mineralization and immobilization, the net amount of mineral N formed depending on the result of these opposing biological processes. Both temperature and pH may be important factors affecting rates of mineralization differentially[25], and if these were markedly different for N and S at, say, different pH levels, they could be important in causing S deficiency.

Other aspects, such as the return of S from the air and in rain, and the leaching of S, need consideration. The only data on the former are those of Gray at Lincoln[33], where on the average 6 lb. S per acre per annum are returned in the rain, and if all this were utilized it would only cover the accumulation of 60 lb. N per acre in pasture soils. Where large quantities of S are returned from the atmosphere, correspondingly less fertilizer S would be needed, depending on the rate of accumulation of soil N. There are good theoretical reasons[11] for supposing that the losses of N by leaching from pastures during the phase of organic-matter accumulation are very small, and the same reasoning should apply to S unless the amounts applied in fertilizers and the natural additions are excessive.

On the face of it, it appears extremely fortunate that superphosphate has been the main phosphate fertilizer used in New Zealand, and whilst the present pattern of farming persists careful investigation is needed before deciding to produce alternative forms of phosphate fertilizers not containing S. Even in areas where trials have shown superphosphate to be inferior to other forms of phosphate, it cannot be inferred that sulphur is not needed, if the Domett situation is at all common. Indeed on some acid soils the superiority of molybdenized superphoshate over superphosphate may be due to the $CaSO_4$-Mn-Mo interaction discussed earlier, and much could be learned from collateral soil and herbage analyses when carrying out field trials. Some liming of acid soils may be unavoidable if maximum yields are to be obtained without raising Mo contents of herbage to dangerous levels, but our trials need observing for another year or two before definite pronouncements can be made on this subject.

Finally, it is clear that considerable care and caution are needed in the design and interpretation of phosphate trials, particularly where different forms of P fertilizers are being compared, or where correlations between soil tests and P responses are being examined. Many of the established views on the relative values of different P fertilizers and P fixation are due for serious revision [34, 3]. These trials suggest that the indiscriminate use of superphosphate (perhaps fortuitously the best fertilizer in most cases) should give way to a more fundamental approach to grassland topdressing. This is particularly so where costs are high in relation to immediate returns, and underlines the need for careful scientific investigation in the initial stage of improvement, which, though complicated, is probably simpler than the subsequent problems of management.

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NOTE ON TUTSAN RUST IN NEW ZEALAND

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Tutsan (*Hypericum androsaemum*) collected in the Wellington district in January 1952 was sent by the Director of the Extension Division of the Department of Agriculture to this Division for identification of a rust attacking this species. Since plants were severely damaged it was suggested that the rust might prove useful in controlling this weed. The rust, *Melampsora hypericorum* (DC) Wint. proved to be a new introduction into New Zealand, although it is widespread in Europe, North Africa, Asia, and North America, where it parasitizes several species of *Hypericum* including St. John's wort (*H. perforatum*).

Within the last two years the rust appears to have spread rapidly through New Zealand, for it has been reported from a number of places between Raglan and Stewart Island.

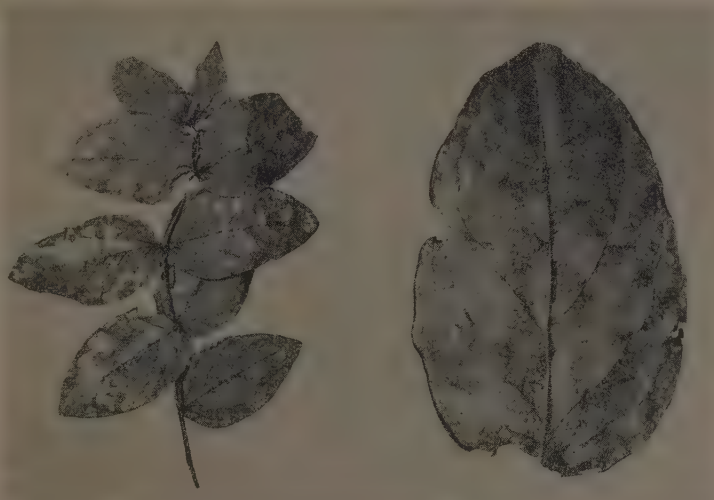

Fig. 1.—Rust-infected tutsan: left, typical blotching of upper leaf surfaces; right, rust pustules (uredosori) on lower leaf surface.

Rust symptoms first appear in late spring or early summer. Characteristic yellow to red irregular blotches appear on the upper surfaces of the leaves, and on lower leaf surfaces golden rust pustules (uredosori), 0.5 to 1 mm. diameter, develop (Fig. 1). At first covered by the leaf epidermis, pustules later erupt, liberating powdery masses of uredospores. Teleutospores have not yet been recorded in New Zealand; overseas they are normally formed during the life cycle of this rust, appearing in late autumn as dark-brown pustules mainly on stems. In our relatively mild climate uredosori can survive through the winter on the limited new growth which is produced on mature tutsan plants and on seedlings. Under favourable conditions uredospores may germinate immediately after being shed from pustules. Thus in one season many generations of uredospores may be formed. As infected host tissues die, the blotched leaf areas turn brown and shrivel. In severe infections the whole plant is defoliated and killed.

Since its introduction last century, tutsan has become a serious pasture weed in New Zealand and it is still spreading, particularly along forest margins. Control is difficult in hill country where cultivation is not practicable. Because of its

extremely rapid spread in the last two years and the damage already caused, tutsan rust might seem a possible means of control. Spores are spread widely throughout the summer and in a few localities large areas of tutsan have been killed. However, in some plants defoliated by rust infection new shoots grow from the base and the plants survive although in a much weakened state. The rust would therefore appear to show promise in providing a partial control of tutsan.

After many years' experience both in New Zealand and overseas however, it has become recognized that fungous diseases whether naturally or artificially disseminated, do not satisfactorily control weeds. Sometimes fungal attack reaches epiphytotic proportions in a localized area. After an initial heavy infection, a balance is usually attained between host and fungus where both exist in certain narrowly fluctuating proportions. With specialized fungi such as rusts, particular combinations of temperature and humidity are essential for germination of spores and infection of plants. It is improbable that these necessary conditions would be continuous over a large area and that rust infection would remain sufficiently severe to exterminate a particular weed. The example of rust of Californian thistle (*Cirsium arvense*) illustrates this.

OESTROGENS AND NEW ZEALAND DAIRY PASTURES

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(Received for publication, 2 December 1954)

Summary

1. Literature concerning oestrogens and other hormones from pasture plants has been reviewed. Evidence of biological activity in most cases has been demonstrated by tests on small animals. Though many effects on farm animals have been suggested, in only two cases has any real proof been obtained.

2. A large number of samples of typical New Zealand dairy pasture, taken at weekly intervals through the period of lush spring growth, have been extracted and assayed for oestrogenic activity. No positive responses were obtained.

3. Lactation curves of cows, maintained under two different types of grazing management on this pasture, showed that a sharp rise in milk production occurred during the period of lush growth.

4. Several samples of subterranean and red clover had a high level of oestrogenic activity. All the activity in red clover plants was in the leaves. A strong biological response was also obtained with a sample of suckling clover.

5. Negative results were obtained from a number of pure samples of grasses, clovers, and other pasture plants.

Introduction

Interest in the oestrogen content of animal feeds was stimulated by the occurrence in Australia of the now classical breeding problem in sheep feeding on subterranean-clover pastures (Bennetts *et al.*[1], Bennetts[2], Schinckel[3]). Extracts of this clover showed oestrogenic activity when tested on laboratory animals (Curnow *et al.*[4], Beck and Braden[5], Robinson[6], East[7]). The variety Dwalganup was the one implicated, but oestrogen has also been found by Robinson[6] in the Mount Barker strain.

Red clover, *Trifolium pratense*, has been found oestrogenic by Bartlett *et al.*[8], Dohan *et al.*[9], and Legg *et al.*[10] These clovers contain oestrogen mainly in the leaves (Robinson[6], Legg[10]) and samples appear to be consistently positive. The isoflavone genistein is the chief source of oestrogenic activity in subterranean clover (Bradbury and White[11]) and the isoflavone biochanin A is an oestrogen of red clover (Pope *et al.*[12]). These two isoflavones and their glycosides are known to occur in other plant species, and some synthetic isoflavones have oestrogenic activity.

Some samples of other legumes are oestrogenic. Lucerne, *Medicago sativa*, was negative[9 10], but one sample was positive[10]; strawberry clover, *Trifolium fragiferum*, gave one positive[6]; *Medicago denticulata* was negative[6], and white clover, *T. repens*, negative[6 10] (Cheng *et al.*[13]).

Some samples of grasses have been found to be oestrogenic, particularly the young plants growing rapidly in the spring. Oestrogens were extracted from rapidly growing spring pasture in England[7], and young grass hastened the sexual maturity of mice (Evans and Evans[14]). Perennial ryegrass (*Lolium perenne*) was oestrogenic in the spring in

the U.S.A.[9], leaves and stems positive especially in the spring in England[10], and leaves, and to a less extent stems, of H1 ryegrass, yielded oestrogen[10]. *Poa pratensis* was negative[9 13], rye (*Secale cereale*) positive, especially in the spring[9], cocksfoot positive[10], negative[9], wheat (*Triticum aestivum*) negative[9], veldt grass (*Erharta calycina*) negative, and timothy (*Phleum pratense*) negative[10].

Hay made from red clover, alsike clover (*T. hybridum*), and white clover, and also lucerne hay, was oestrogenic[13]. Maize and wheat grains were negative[13], rice and wheat grains oestrogenic (Fellner[15]), corn negative, sprouted oats positive (Walker and Janney[16]), soya bean meal was negative[13], but the glycoside genistin from the bean was oestrogenic[17].

A great number of plants, many of horticultural use, and a range of organic substances, have been shown to be oestrogenic when tested on laboratory animals or occasionally when chemically assayed. In a few instances the oestrogen has been isolated and identified with an animal steroid hormone. Costello and Lynn[18] obtained oestrogen having the absorption spectrum of oestriol from licorice root, Butenandt and Jacobi[19] isolated oestrone from palm-kernel residue, Hassan and Wafa[20] isolated an oestrogen (probably oestrone) from date-palm pollen, and Skarzynski[21] isolated oestriol from pussy-willow flowers. A pure oestrogen different from the known animal ones was isolated from roots of *Butea superba* by Butenandt[22] and Schoeller *et al.*[23].

The Australian problem was the first instance of a marked effect of oestrogens in food on farm animals. In another instance when unusual lactational phenomena occurred in grass-fed dairy cows, oestrogen in food was demonstrated by chemical assay (Schoop and Klette[24]), but was considered to originate from manure. Coussens and Sierens[25] reported that a war-time diet of tulip bulbs caused aberrations in the reproductive cycle of Dutch women. Tests with mice showed that the bulbs had oestrogenic activity. In some cases the effects of oestrogens in pasture on fattening lambs (Cheng[13]) or on lactating cows (Bartlett[8]) have been inferred, though there is yet no direct proof of the hypotheses.

There are few references to gonadotrophic effects of plant extracts. This idea does not seem to have been pursued since the publications of Friedman *et al.* [26 27] on gonadotrophic effects of immature oat grass, Sudan grass, and corn plants. On the other hand, the plant *Lithospermum ruderale*, which has a gonad-suppressing effect, has received considerable attention lately (Zahl[28], Drasher[29]) also *L. officinale* which has similar action (Wiesner and Yudkin[30]).

No record has been found of androgenic or progesterone-like activity in non-animal extracts (Butz and Fraps[31], Zondek[32]) and one sample of our pasture gave negative results for androgen. A positive Zimmermann reaction for ketosteroids was taken to indicate possible androgen in *Chrysactinea mexicana* (Lappas and Gustafson[33]).

In view of the many suggestions in the literature that the galactopoietic effect of rapidly growing spring pasture is due to its oestrogenic activity, it seemed pertinent to investigate typical New Zealand dairy pasture for the presence of such substances.

Cunningham and Hogan[34] have found a high oestrogen content in many samples of subterranean clover, and an appreciable content in

some samples of red clovers growing in New Zealand, traces in very early spring growth of perennial and short-rotation ryegrasses, and none in white clover and many other grass samples. An occurrence of lactation in wethers was ascribed to oestrogen in the subterranean and red clovers in the pasture.

MATERIAL AND METHODS

Two adjacent paddocks of the same soil type were selected. Paddock A (three acres) was part of a rotationally-grazed farm while B (fifteen acres) was the night paddock of a set-stocked farm of the same total area and carrying capacity. Both paddocks contained typical good-quality dairying pasture.

Analysis of the botanical composition of the two paddocks at each collection was made on 20g. of pasture. The predominant species were perennial ryegrass (42% to 53%) and white clover (19% to 21%), with less cocksfoot (16% to 5%), Yorkshire fog (10% to 6%), *Poa trivialis* (7% to 5%), and up to 3% of *Poa pratensis, P. annua,* browntop, sweet vernal and paspalum; less of other species of grasses and clovers, and 4% to 2% of flat weeds, buttercup, etc. The first figure refers to paddock A, the second to paddock B, values being means of many samples. There was little change in composition of either area during the experiment. Paddock B was more unevenly grazed. Paddock A was grazed eight times during the course of the experiment, while paddock B was grazed every night. There were fifty cows in each herd, average calving time being similar in each.

Milk and fat production were recorded throughout the period.

Observations for the present study began on 11 August when pasture growth was relatively dormant under winter conditions.

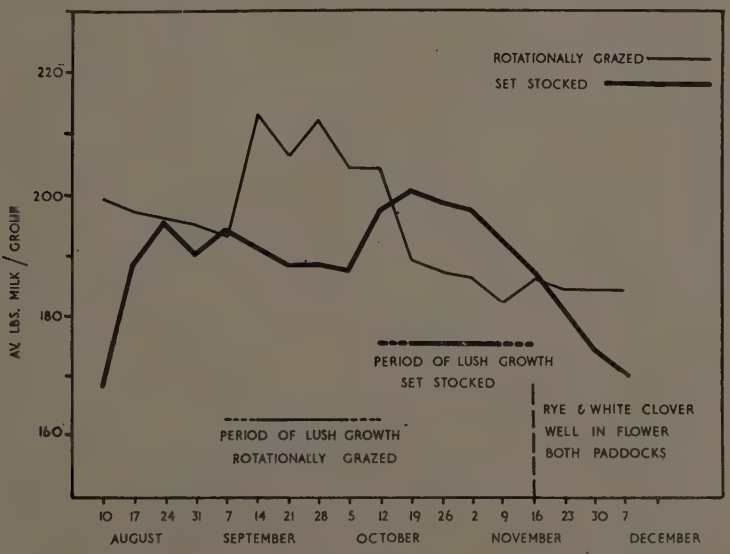

Fig. 1.—Milk production (lb.) related to periods of lush pasture growth (1949) spring.

Collections were made thereafter at weekly intervals until 8 December, when most of the pasture species were at the late flowering stage. The date of appearance and period of lush growth for each paddock were noted.

To ensure unbiassed sampling, three 1/1,000 ac. frames were distributed in each field. All the grass from under each frame was removed by shears at each day of collection and the frames then moved to fresh positions. Samples of 200g. (wet wt.) were taken at random from the bulked collection for each paddock and preserved in 95% alcohol. In addition, samples were obtained of various species of grasses, clovers, and other plants commonly found in pastures grazed by stock (Table 1).

The procedure for extraction was Beck's modification (pers. comm.) of Robinson's[6] method for subterranean clover. The resulting extracts were taken up in 10 ml. of peanut oil and assayed for oestrogenic activity by the method of Evans *et al.*[35] Four mice were used for each test. Each mouse received the equivalent of 7g. wet weight of pasture. Positive results were obtained on several locally collected samples of subterranean clover subjected to these procedures.

TABLE 1. SPECIES TESTED (NOVEMBER–DECEMBER 1949) WITH NEGATIVE RESULTS

Botanical Name	Common Name	State of Growth	Wet Wt. (g.)
Anthoxanthum odoratum L.	Sweet vernal	Early flowering	100
Holcus lanatus L.	Yorkshire fog	Flowering	100
Dactylis glomerata L.	Cocksfoot	Flowering	150
Poa trivialis L.	Rough meadow grass	Early flowering	120
Festuca arundinacea Schreb.	Tall fescue	Early flowering	200
Paspalum dilatatum Poir	Paspalum	Early growth	150
Festuca rubra L. var. *compacta* Gaud.	Chewing's fescue	Flowering	180
Lolium multiflorum Lam.	Italian ryegrass	Leaves, etc.	200
Cynosurus cristatus L.	Crested dogstail	Flowering	80
Sonchus oleraceus L.	Sow thistle	Flowering	200
Hypochoeris radicata L.	Catsear	Flowering	200
Taraxacum officinale Weber	Dandelion	Flowering	200
Ranunculus sardous Crantz	Hairy crowfoot	Flowering	200
Rumex crispus L.	Curled dock	Flowering	200
Plantago lanceolata L.	Ribgrass	Flowering	184
Trifolium filiforme L.	Lesser suckling clover	Flowering	28
T. campestre Schreb.	Hop trefoil	Flowering	83
T. arvense L.	Haresfoot trefoil	Flowering	200
T. glomeratum L.	Clustered clover	Flowering	200
T. repens L.	White clover	Flowering	200
T. hybridum L.	Alsike clover	Flowering	162
T. resupinatum L.	Reversed clover	Flowering	23
T. tomentosum L.		Early pod	80
T. fragiferum L.	Strawberry clover	Flowering	100
Lotus hispidus Desf.	Hispid birdsfoot trefoil	Flowering	155
L. uliginosus Schkuhr.	Greater birdsfoot trefoil	Pre-flowering	200
Medicago sativa L.	Lucerne	Pre-flowering	200
M. lupulina L.	Black medick	Flowering	200
Melilotus indicus (L.) All.	King Island melitot	Flowering/pods	80
Vicia angustifolia L.	Common vetch	Flowering/pods	200
V. tetrasperma (L.) Moench.	Smooth tare	Flowering	200

RESULTS

The lactation curves for each of the two groups of cows grazing on the set-stocked and rotationally grazed farms are shown in Fig. 1. The periods of lush growth on each of the two different paddocks are also shown. It can be seen clearly that there was a difference in the time of spring flush of grass according to the systems of grazing management, the set-stocked paddock coming away nearly a month later than the rotationally grazed one. The peak in production of each herd coincided with the period of lush growth in each paddock.

No positive oestrogenic response was obtained from any of the weekly samples of pasture from these two paddocks.

Concurrently, extracts from pure samples of nine different grasses of varying agricultural importance collected in the district gave negative results, as did extracts from various pasture weeds (Table 1).

Strong reactions were, however, obtained from three samples of subterranean clover in early-flowering or flowering stages obtained from localities outside of the experiment (Table 2). Positive results were also obtained from samples of red clover at several different stages of growth, from the very young leaf to the flowering stage. Though a strictly quantitative result is not obtainable from the small numbers of mice used in each assay, results indicated that the oestrogenic potency of the red clover samples was at least as great as that from subterranean clover. At Ruakura, six ewes were grazed for some time on a practically pure sward of red clover, having a high oestrogen content. During this time they were successfully mated, though the urinary oestrogens were

TABLE 2. OESTROGENIC ACTIVITY IN CLOVERS

Description	Time of Year	Wet Weight Sample (g.)	Mean Uterine Weight in Treated Mice (mg.)	Mean Uterine Weight in Controls (mg.)	Approx. μg. Oestradiol	
					Whole Sample	100 lb. D.M.
T. subterraneum						
Almost flowering	Oct.	150	16.5	9.3	1.7	7.5
Early flowering	„	200	24.2	9.3	2.7	9.0
Flowering	„	118	strongly positive			
T. pratense						
Young leaf stage						
After heavy grazing	Dec.	100	20.5	9.5	2.0	13.3
Pre-flowering	Nov.	100	17.0	6.4	1.7	11.3
Pre-flowering	Mar.	100	20.0	11.0	2.0	13.3
Parts of Plants:						
Roots	Jan.	70	negative			
Lower stems		100	„			
Top stems		100	„			
Flowering heads		100	„			
Leaves		100	32.5	11.0	4.0	27.0
T. dubium						
Flowering	Oct.	80	strongly positive			
Flowering	Nov.	67	negative			
Late flowering	Dec.	200	„			
Early growth	Sept.	90	„			
	Sept.	90	„			

appreciably higher than for corresponding animals on pasture (Bassett *et al.*[36]). Assays on extracts from various components of red clover plants showed that all the activity was concentrated in the leaves. A strongly positive response, comparable with that from subterranean, was also obtained from one sample of suckling clover, but four others were negative.

The uterine weights of the mice were not compared with reactions to standard oestrogen at the time of the experiment. However the responses of the same stock of mice to oestradiol were recorded shortly afterwards. For comparative purposes the approximate equivalent for each sample has been determined. Work at Ruakura has shown that the dry-matter content of young leafy growth can be estimated as about 15 %. The oestradiol equivalent of 100g. of dry matter has thus been calculated.

DISCUSSION

The results obtained with subterranean and red clovers showed that it was possible to extract and assay the oestrogenic material in plants by the chemical and biological methods used here. In fact, the values for red clover, expressed as μg. oestradiol per 100 g. plant dry matter, were higher than those found by the English workers (Legg[10]). It is, of course, possible that the use of much larger samples of pasture might have revealed the presence of extremely small amounts of oestrogens. However, as the test mice received (by injection) the equivalent of their body weight in wet weight of grass in three days, and a dairy cow might be expected to eat approximately one-third its body weight in the same period of time, it is not likely that the hormone would be present in sufficient amounts to have any galactopoietic effect on the grazing animal. Oestrogens could have been present for a very short period in the rapidly growing pasture and thus not have been detected in the weekly sample, but even in this event it is unlikely that such a brief period of oestrogenic activity could be of any significance to the farm animal.

On the other hand, it has been shown clearly that the peak in lactation of grazing cows is closely associated with the period of lush growth in spring pasture though oestrogens were not present. Thus it would seem that, at least as far as New Zealand cows and pastures are concerned, factors other than hypothetical grass oestrogens must be responsible for the stimulating effect on milk yield of spring grass.

Evidence from various sources reveals that sudden changes in feed can have quite marked effects on farm animals. The well known practice of flushing breeding ewes to increase ovulation rate operates through gonadotrophic stimulation. It is possible that such phenomena, associated with increased pasture supply, could be due to an increase in endogenous oestrogens produced by stimulated ovaries of the animals themselves.

In view of the findings of the present investigation and the lack of proof of the various effects which have been postulated on farm animals, it is unfortunate that such postulates have been taken up more uncritically than the original authors no doubt intended. In many reviews and discussions the implication of oestrogens in both adverse and beneficial effects on farm animals has become almost an accepted fact. It is worth noting that though oestrogens have been found in potatoes[16], honey[37, 38], and other common human foods, no associated oestrogenic effects have been suggested for man.

Recent observations at Ruakura on two lots of calves run on each of the rotationally grazed and set-stocked farms are of interest in this connection. During a severe autumn drought the set-stocked calves were on almost a starvation ration compared with those that were rotationally grazed. Heavy rain and warm temperatures caused a marked flush of rapidly growing grass on both areas. Teat lengths of the set-stocked calves increased suddenly by up to 15 % during one week, while there was no such change in the figures for the other group (G. M. Elliott, pers. comm.). There was no associated change in body weight. Six samples, each of 100 g. of dried grass, collected from several different areas during this autumn flush were extracted and assayed for oestrogens by the methods already described. No positive responses were obtained.

Acknowledgements

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EXPANSION AND STRUCTURE OF THE NEW ZEALAND PEDIGREE AYRSHIRE BREED, 1910 TO 1950

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(Received for publication, 5 October 1954)

Summary

1. The pedigrees of heifers registered in the Ayrshire herd books of New Zealand and representing births at five-year intervals from 1915 to 1950 have been analysed.

2. The contributions of importations to the breed in each of the years sampled have been shown. Approximately 72% of the genes in the heifers registered in the 1950–51 herd book were derived from animals imported since 1910 and about 30% from animals imported since 1936.

3. Certain imported animals have made large individual genetic contributions to the breed. Three sires used in one herd have together contributed 23% of the genes present in the 1950–51 heifer registrations.

4. The analysis of the breed according to the function of the herds comprising it indicated that the Ardgowan herd contributed almost one-third of the genes in the sample chosen from the 1950-51 herd book. A further five herds made much smaller but approximately equal contributions of about 5 to 6% to the same sample.

5. A figure has been included to show the varying genetic contributions of some individual herds to the breed during the period 1925–1950. The popularity of the more important herds has been closely associated with the use of imported sires.

Introduction

In the absence of native breeds of cattle, New Zealand has relied entirely on importations in founding its present cattle population. The first cattle were introduced in 1814 from Australia, and importations have continued from that time. In 1848 the first Ayrshire bull was imported from Scotland and it was the first representative of the specialized dairy breeds to be introduced into the country. Despite this early introduction, the Ayrshire breed now ranks only fourth in popularity among the breeds used for dairying but, with an increasing proportion of the total milk produced in the Dominion being used for the liquid milk trade, the Ayrshire breed may assume a more important role in the future. Further, the breed offers certain interesting aspects for study and it is the purpose of this paper to trace briefly the development of the Ayrshire breed in New Zealand and to indicate its functional structure in order to provide background information which should be of interest to breeders and geneticists alike.

Material and Methods

Since the pedigrees of registered animals provide the most authentic information available, this study has been confined to data derived from the Ayrshire Herd Books of New Zealand, the first volume of which was published in 1910. Early volumes contained the particulars of many

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animals born during the two decades prior to 1910. Thus the first phase of the study was to list all the females registered in Volumes I–IX according to their year of birth. Samples of 100 heifers were then taken from the registered heifers born in 1915 and 1920. The remaining samples of 100 heifers representing female registrations at five-yearly intervals from 1925 to 1950 were drawn from the single herd books in which the majority of registered heifers born in a sample year were registered. The individuals comprising each sample were chosen at regular intervals from the herd books according to the number of animals registered in the herd book sampled. The sampling varied from every second registered female born in 1915 to every thirtieth female registered in the 1950–51 herd book.

For each individual in the eight samples of 100 registered heifers, a complete pedigree was constructed to the eight great-grandparents, and then one line was traced at random from each great-grandparent until an imported animal or an animal born in New Zealand before 1910 appeared. The herds in which animals appearing in the pedigree were bred were also noted.

BREED SIZE AND POPULARITY

Though ranking fourth in total cow numbers in New Zealand, the Ayrshire breed at present has the second largest number of annual heifer registrations. The 3,300 heifers registered in the 1951–52 Ayrshire Herd Book numbered about one-sixth of the Jersey registrations but exceeded the Friesian registrations by approximately 400 in the same year. Annual registrations of both males and females from 1910 onwards are shown in Fig. 1. The apparent increase in registrations born in

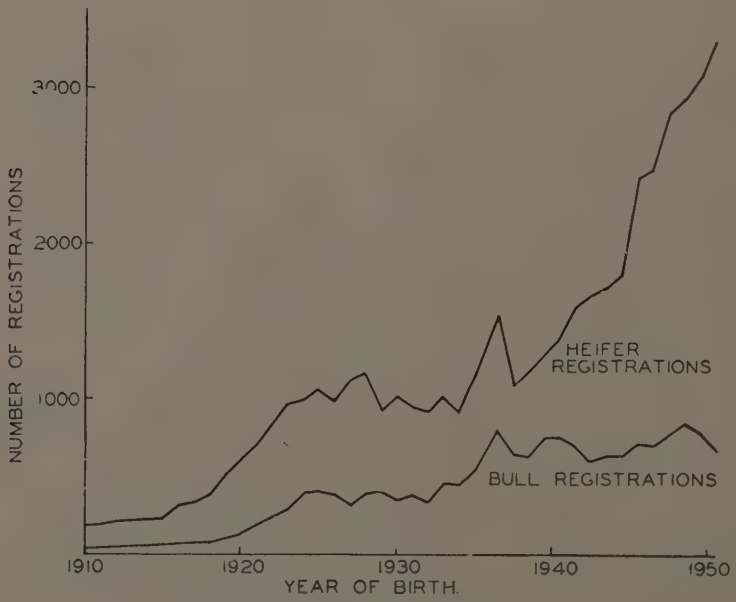

Fig. 1.—Male and female Ayrshire registrations, 1910–50.

1936-37 is due to an alteration in registration procedure and represents births during a period of more than one calendar year. The two periods of rapid expansion, 1915-25 and 1938–50, are similar to though less accentuated than those for the Jersey breed in the Dominion during the same period (Stewart, 1952). The failure of male registrations to increase during the latter period of expansion is noteworthy and is probably due to the high proportion of pedigree cattle in the Ayrshire breed as a whole. The total number of Ayrshire females—pedigree, grades, and crossbreds—over one year of age at 31 January 1950 was approximately 73,200 (N.Z. Year Book, 1950). The average number of pedigree Ayrshire bulls registered annually during the period 1947–49 was 800. Assuming that each bull has a herd life of three years, and serves on the average twenty-seven females each year (N.Z. Dairy Board, 1951), and that there is little crossbreeding, then, of all cattle classed as Ayrshire, probably about 80% are mated to pedigree Ayrshire bulls. Comparable figures for Jersey and Friesian cattle are 38% and 45% respectively. The demand for registered Ayrshire bulls is almost satisfied, and the recent rapid increase in the numbers of registered Ayrshire heifers does not represent increased popularity of the Ayrshire breed of cattle—grade and pedigree—but rather an increase in the proportion of the breed that is registered. This is borne out by official statistics. In 1921, Ayrshire cows comprised 3.0% of the national dairy herd, and in 1950, 3.1%. During the same period, Ayrshire heifer registrations increased more than fourfold while the national herd was approximately doubled.

EFFECT OF IMPORTATIONS

The influence of importations on the Ayrshire breed in New Zealand is of particular interest at present in view of a recent report (Donald, Deas, and Wilson, 1952) on the widespread occurrence of dropsy in Scottish pedigree Ayrshire herds, a condition which appears to have reached its present high incidence in less than thirty years. A dropsical calf was first reported in New Zealand Ayrshires by Hancock (1950), and during 1953 a number of cases, particularly in the south Auckland area, have been recorded (New Zealand Dairy Board, pers.

TABLE 1. SOURCE OF AYRSHIRE IMPORTATIONS INTO NEW ZEALAND, 1910–49

Date of Importation	Source of Importation					
	Scotland		Canada or U.S.A.		Australia	
	Bulls	Cows	Bulls	Cows	Bulls	Cows
1910–15	14	15	—	—	2	—
1916–25	7	2	1	—	1	—
1926–35	2	—	14	—	—	—
1936–45	8	1	5	2	1	17
1946–49	7	4	5	—	—	2
Total	38	22	25	2	4	19

comm.). It seems likely that one or more imported animals carried the single recessive gene which gives rise to dropsical calves and that this gene is becoming increasingly frequent in the Dominion.

In constructing the eight-line pedigree described above, the important genetic contribution made by imported animals to the breed was immediately apparent. Lists of animals imported into New Zealand since 1910 were compiled and the contribution made by each animal to the eight samples of 100 heifers drawn from every fifth year was calculated by a modification of Wright's short method described by Robertson and Asker (1951).

In Table 1 the Aryshire animals imported into New Zealand since 1910 have been distributed according to the country in which they were born. Scotland has been the main source of importations with Canada assuming greater importance during a period when quarantine regulations did not permit the direct entry of cattle from the United Kingdom. These Canadian-bred animals were nearly all derived from parents or grandparents born in Scotland. During the period 1910–15 males and females were imported in approximately equal numbers, but since 1916, with the exception of a relatively large importation of females from Australia in 1941 by a single breeder establishing a herd, the emphasis has been on male importations.

Imported animals were grouped according to their date of entry into New Zealand. Since 1910 there have been more or less distinct

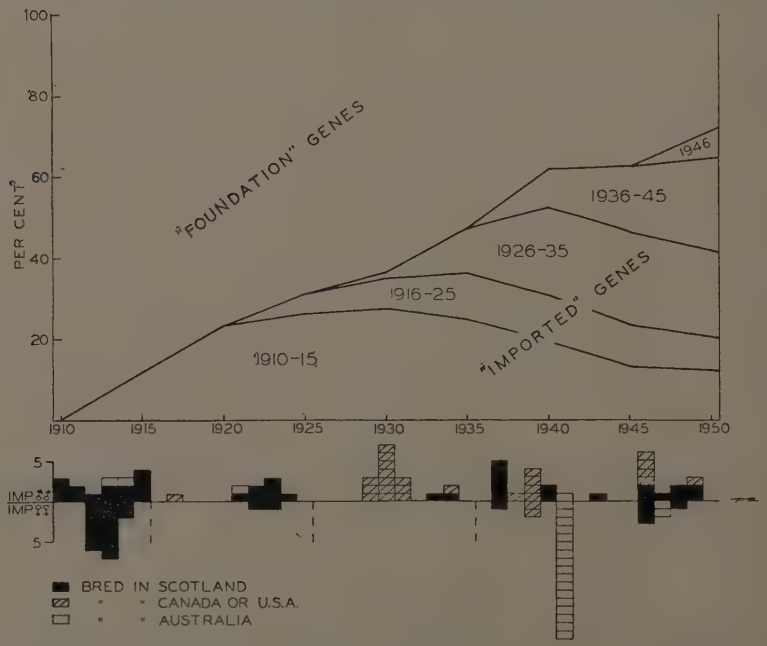

Fig. 2.—The genetic contributions of importations since 1910 to samples of 100 heifer registrations representing registered births at five-yearly intervals, 1915–50.

groups of imports: 1910–15, 1916–25, 1926–35, 1936–45 and 1946–49. These import groups are shown in Fig. 2 together with their genetic contribution to each of the eight samples of 100 heifer registrations. The contribution of each animal to any sample is measured by the number of lines of the total of 800 which trace back to it. The genetic contribution made by animals imported since 1910 has been steadily increasing, and in the sample of heifers born in 1950 the following genetic contributions have been made by all animals imported since the dates specified:

6.5% of the genes derive from 12 males and 6 females imported since 1946.

29.1% of the genes derive from 26 males and 26 females imported since 1936.

52.0% of the genes derive from 42 males and 26 females imported since 1926.

59.9% of the genes derive from 51 males and 28 females imported since 1916.

72.0% of the genes derive from 67 males and 43 females imported since 1910.

In view of the increasing incidence of dropsical calves in New Zealand, it is interesting to note that more than half of the genes in the

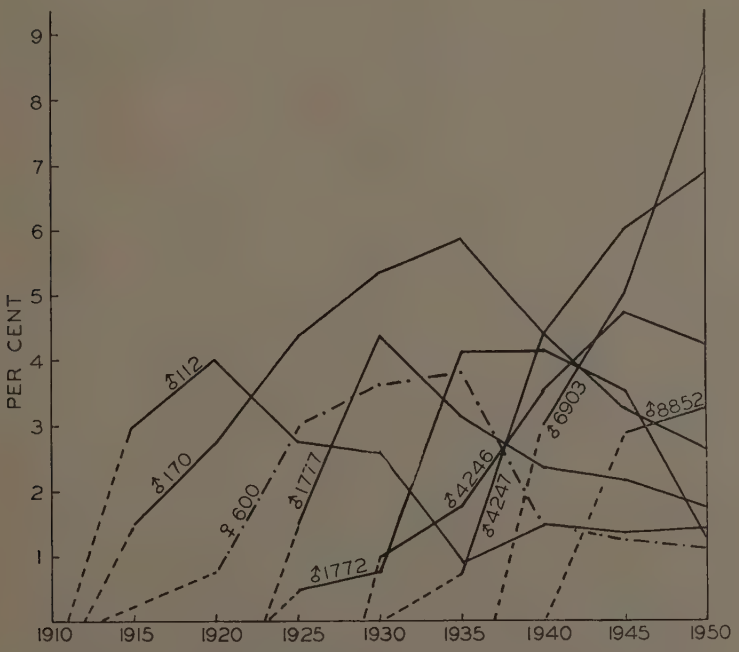

Fig. 3.—The direct relationship of the more prominent imported animals to eight samples of 100 heifer registrations representing registered births at five-yearly intervals, 1915–50.

1950 sample are derived from imports since 1926, the period during which the gene causing the dropsical condition has become increasingly frequent in Scottish Ayrshires (Donald *et al.*, 1952).

The individual contributions of certain imported animals are also of interest. The more important of these have been chosen, and their direct relationship to each of the eight samples is shown in Fig. 3. Though only three animals have had a direct relationship of more than 5% with one or more samples, in later years particularly, a relatively few animals have together made very large genetic contributions to the breed. For example, in the 1950 sample, 23% of the genes derived from three sires, all of which were used in the one herd. The rate at which the genes of the more important animals have been spread through the breed is, in the absence of artificial insemination, remarkably high. Sire 6903 was born in 1936, brought to New Zealand the following year, and used widely in a large herd. Twelve years after his first offspring was born in the Dominion, Sire 6903 had a direct relationship of 8.5% to the breed. In other words, one gene in every twelve of the 1950 sample derived from Sire 6903. Only one female, Dam 600, imported in 1912, has made significant contributions to the different samples, and this dam's importance is due mainly to the widespread demand for the progeny of two of her sons.

The rate at which the influence of the important animals shown in Fig. 3 has been spread is remarkably uniform throughout the period sampled despite changes in breed size. This is due to the relatively few herds that have used imported animals dominating the breed. This aspect will be discussed in a later section.

Breed Structure

Functional stratification of the herds comprising a breed depends primarily upon the recognition by breeders of the superiority, real or imagined, of one or more of their contemporaries. Animals from these "superior" herds—usually bulls—are generally in great demand by breeders, while breeding stock from "inferior" herds is not widely distributed among pedigree herds. Thus, a herd may fall into one of the following categories, depending on the fate of the bulls bred in it:

(1) Herds that register bulls used in other pedigree herds.

(2) Herds that register bulls used only in the pedigree herds in which they were bred.

(3) Herds that register bulls not used in any pedigree herds.

(4) Herds that do not register bulls.

Bulls bred in all four categories of pedigree herds may be used in grade herds. The first group of herds may be called "breeders' herds" and the latter three grouped together as "multipliers' herds." The only real difference among the categories forming the "multipliers' herds" is that the multiplication process differs in intensity.

The 1950–51 Ayrshire herd book was analysed by the method described by Robertson and Asker (1951) and the following structure was found to obtain (Fig. 4). Of the 375 herds from which heifers were registered various strata were identified consisting of:

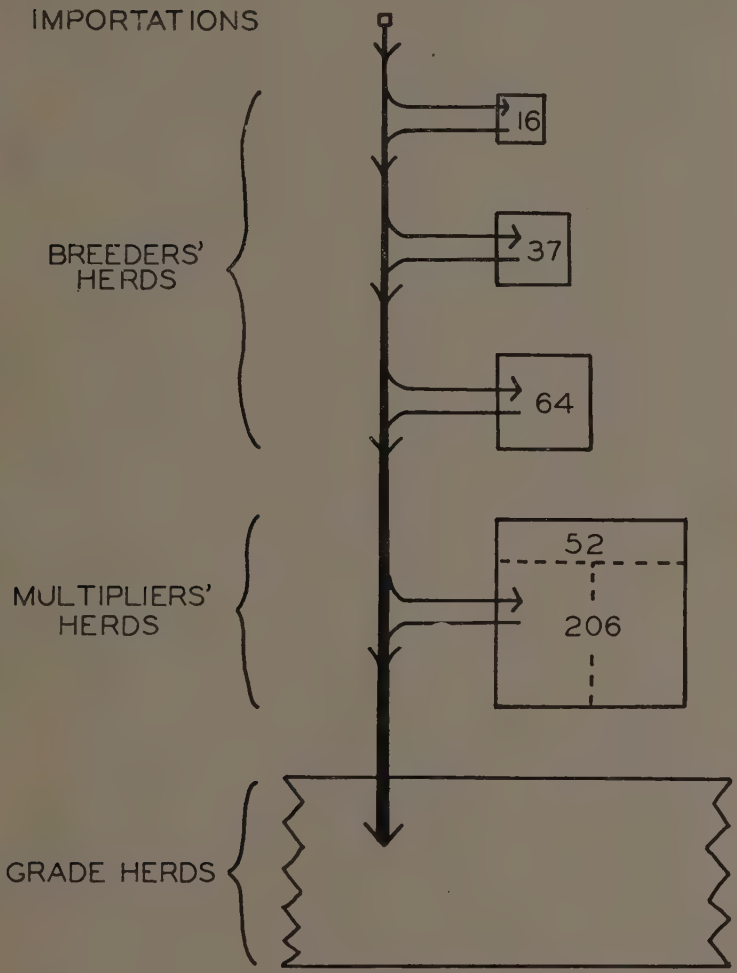

Fig. 4.—The structure of the New Zealand pedigree Ayrshire breed, 1950.

(a) 107 herds that did not register males in the 1950–51 herd book;

(b) 99 herds that though registering males were not represented among the sires of registered heifers;

(c) 52 herds that bred bulls used only in the herds in which they were bred.

These 258 herds comprised the "multiplying herds" of the breed.

(d) 117 herds that bred bulls used in other pedigree herds.

These 117 herds comprised the "breeding herds" of the breed.

In order to determine the relative importance of these "breeders' herds", a method described by Wiener (1953) was employed. Animals appearing in the eight-line pedigrees described previously were coded to show the herds in which they were bred. If in one sample of 100 registered heifers each heifer was by home-bred parents, which in turn were by home-bred parents, and so on, the genetic contribution of a herd to the sample would be proportional to the number of heifers bred in that herd that appeared in the original sample. More than 80% of Ayrshire heifers registered are by purchased sires and approximately 40% are out of purchased dams. The herds in which these purchased animals were bred have obviously contributed to the sample though the more generations separating an animal born in a particular herd from the individual in the sample the smaller the genetic contribution. The total number of appearances of a herd in the pedigrees does not, however, represent the genetic contribution of that herd since the appearance of a herd in one generation of a pedigree may be the cause or result of its appearance in the next generation in the same pedigree. Thus the contribution of each herd depends upon the number of independent occurrences in each pedigree and the generation in which each occurs. In a sample of 100 heifers, one occurrence of a herd in the parental generation will mean that the herd has contributed 1/200 of the genes in the sample or one half of one per cent. Should one of this animal's parents be born in the same herd, the herd will also appear in the grandparental generation, but since its appearance is not independent of its occurrence in the parental generation, the herd makes no further contribution. Should the other grandparent in the same half of the pedigree be born in a second herd, this herds' contribution to the sample will be 1/400 or one quarter of one per cent, and so on.

Table 2 shows the individual and combined contributions of the six most important herds and importations since 1910 to the sample of 100 heifers drawn from the registrations born in 1950. Only the con-

TABLE 2. PERCENTAGE GENETIC CONTRIBUTION OF SIX MOST IMPORTANT INDIVIDUAL HERDS AND IMPORTATIONS SINCE 1910 TO THE PEDIGREES OF THE SAMPLE OF 100 REGISTERED AYRSHIRE HEIFERS BORN IN 1950.

Herds	Total Genetic Contribution (%)	Generation				
		1	2	3	4	5 and sub.
Ardgowan (D1)	31.5	8.0	12.5	8.1	2.9	
Burnside (H3)	5.1	4.0	0.8	0.1	0.2	
Carmel Glen (T2)	6.6	1.0	2.0	2.2	1.4	
Dalemere (H1)	5.5	2.5	2.0	0.9	0.1	
Glenhope (B1)	6.5	3.0	2.5	0.4	0.6	
Puketutu (K4)	6.0	6.0	0	0	0	
Six herds	56.6	24.5	17.2	10.5	4.4	
Importations since 1910	72.0	3.5	12.2	13.5	13.4	29.4

tributions made during the last four generations were considered, partly because herds almost always have a limited duration and those herds that were of immediate importance to the breed in 1950 as bull-breeding herds were of relatively minor importance fifteen to twenty years previously, and partly because the contribution of herds that were important several generations previously becomes greater further back in the pedigrees. If the latter herds were included, as would be the case if many more than four generations were considered, the total genetic contribution made by all the selected herds could be considerably in excess of 100% of the genes existing in the 1950 sample. For example, if all the eight hundred lines of the one hundred pedigrees considered for one year ultimately traced back through various herds to one particular herd, that herd would have contributed 100% of the genes in the bred in the year sampled. In the same sample another herd may have supplied half the sires in the parental generation and it would have contributed through these sires 25% of the genes in the sample. While this does not invalidate the method, the effect of taking many generations into consideration would be to give the relative importance of herds during the period for which authentic pedigree details were available, whereas interest is centred more on the absolute contributions of individual herds at a particular time.

The importance to the Ayrshire breed in the Dominion of the Ardgowan herd may be seen from Table 2. Almost one-third of the genes in the sample have come through this herd in the last four generations. A further five herds made considerably smaller but approximately equal contributions to the sample. It is of interest to note that the Puketutu herd, founded in 1941 largely from imported animals, achieved prominence in less than ten years. The remaining "breeders' herds" were relatively unimportant.

The total genetic contribution of the six herds combined (56.6%) does not equal the sum of their individual contributions (61.2%) since

TABLE 3. HERDS GROUPED ACCORDING TO THE AVERAGE NUMBER OF REGISTERED MALE PROGENY DURING A THREE-YEAR PERIOD OF THE BULLS THEY HAVE BRED (1946–47 AND 1948–49 HERD BOOKS).

Number of registered bulls by sires bred in any one herd (3-yr. average)	Percentage of all "breeders' herds"	Percentage of all herds registered in 3-yr. period	Percentage of all bulls registered in 3-yr. period	Herds represented
150–	0.8	0.2	28.4	D1.
100–149	1.6	0.5	11.3	B1, H1.
50–99	2.3	0.7	9.8	B21, H3, T2.
40–49	3.0	0.9	9.3	K4. . . .
30–39	5.3	1.7	12.2	
20–29	4.5	1.4	7.8	
10–19	7.5	2.4	7.7	
5–9	14.4	4.5	6.2	
2–4	31.1	9.7	5.2	
1	29.5	9.2	2.0	
Total No. for 3-yr. period	132	424	1963	

to some extent animals bred in these herds are used by one or more of the other five herds. The small difference, however, indicates that bull transfers among these six herds have not been frequent owing to each of them having relied mainly on imported bulls for the introduction of new "blood." The genetic contribution of 52% made by importations during the four to five generations that have elapsed since 1926 is channelled to a considerable extent through these six herds.

To confirm the estimates shown in Table 2, herds were grouped according to the average number of registered male progeny during a three-year period (1946–7 to 1948–9) of the bulls they bred. The number of registered male progeny of bulls bred in one herd will depend upon the number of its bulls used in pedigree herds and the extent to which the individual bulls were used. The results are shown in Table 3. Of the 424 Ayrshire breeders who registered animals during the three-year period, only the bulls bred in 132 herds sired the 1963 registered males by New Zealand-born sires. Almost 50% of the registered males were sired by bulls bred in six herds, five of which correspond to those shown in Table 2. The Ardgowan herd (D1) supplied the sires of 28.4% of the males registered during the three-year period. In the five herd books published between 1947 and 1951 the percentage of registered bulls by Ardgowan sires was 31.1%, 29.7%, 24.5%, 28.2%, and 23.2%. These findings indicate that only a small proportion of the Ayrshire breed is of real significance, and that among this small group of herds the Ardgowan herd is pre-eminent.

During the ten to fifteen years preceding 1950, it is clear that certain herds have enjoyed considerable popularity. The genetic contributions of these herds to the breed have not been uniform, and the changing significance of herds in the breed and the reasons underlying such changes are of interest. The influence of the more important herds on the samples of 100 registered heifers was measured in the manner described by Wiener (1953). Only the samples representing births at five-yearly intervals from 1925 to 1950 were studied, since the herd in which many of the ancestors of animals born in 1915 and 1920 were bred was not always clear from the extended pedigrees published in the early volumes of the herd books. The way in which the importance of an individual herd waxes and wanes is clear from Fig. 5. The Ardgowan herd (D1) achieved its present pre-eminence during the period subsequent to 1935 and its importance appears to be still increasing. The Ivanhoe (W1) and Waipapa (M1) herds, though still registering animals, have declined in importance since the 1930s when together with the Government Farm herd (M8) at Moumahaki, now defunct, they dominated the breed. The Carmel Glen herd (T2) has been of lesser but fairly uniform importance during the period studied, while the Gowan Bank (P4) and Porirua (P5) herds have declined to negligible importance in the 1945 and 1950 samples. The Bonny Glen (B2), Dalemere (H1) and Puketutu (K4) herds have become more important in recent years though the influence of the former appears to be waning.

The years in which imported sires have been introduced into these herds are also shown in Fig. 5. Almost without exception the period during which a herd made relatively large genetic contributions to the samples followed the use of one or more imported sires. Clearly one of the reasons for Ayrshire herds becoming fashionable in the Dominion

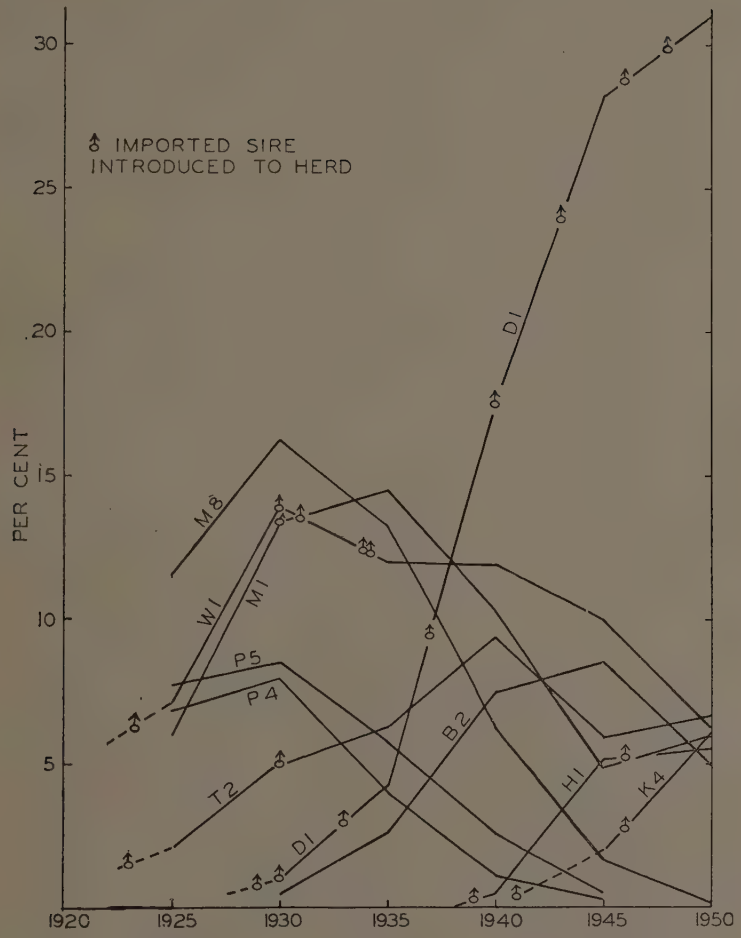

Fig. 5.—The genetic contributions of the more important herds to samples of 100 registered heifers representing registered births at 5-yearly intervals, 1925–50.

is associated with the use of imported sires. This is confirmed from a comparison of the genetic contributions of the Ardgowan herd to the breed since 1930 with that of the eight imported sires used between 1929 and 1949 in the Ardgowan herd (Table 4). The popularity of this herd is clearly associated with the popularity of the imported sires that have been used.

Discussion

There are no means available for determining whether the genetic changes due to the preference of breeders for imported animals and their

TABLE 4. THE DIRECT RELATIONSHIP OF IMPORTED SIRES USED IN THE ARDGOWAN (D1) HERD TO THE AYRSHIRE SAMPLES 1930–50.

Name of Sire	Year Imp.	Direct Relationship to Samples				
		1930	1935	1940	1945	1950
Thorncroft Star Boy (4246)	1929	1.0	1.8	3.5	4.8	4.2
Avonsyde Star of Fame (4247)	1930		0.8	4.4	6.0	6.9
Hobsland Prince of Perfection (5383)	1933			1.0	1.5	1.0
Netherton Mahmoud (6903)	1937			3.0	5.0	8.5
Harleyholm Topsman (8852)	1940				2.9	3.2
Lessnessock Twinkle (10814)	1943				0.5	2.0
Netherton Typhoon (12806)	1946					0.8
Willoxton Lustre (14351)	1948					0
Total genetic contribution		1.0	2.6	11.9	20.7	26.6
Genetic contribution of Ardgowan-bred animals		1.0	4.3	17.5	28.3	31.5

offspring have resulted in any genetic improvement of productive qualities in the breed as a whole, but it is clear from the foregoing analysis that the genetic material in a small breed may be changed relatively rapidly even in the absence of artificial insemination. This is a direct consequence of the functional stratification of herds within breeds due to the real or imagined superiority of certain animals and herds in the opinions of contemporary breeders.

Herds that have made relatively large genetic contributions to the New Zealand Ayrshire breed have shared a preference for imported sires. These sires have in the past been obtained from the most popular herds in Scotland, and it is likely that the Ayrshire breed in New Zealand contains all the genes of the Scottish Ayrshires, both desirable and otherwise. There would thus appear to be ample opportunity for breeders in the Dominion to adopt constructive breeding policies of their own rather than rely on Scottish sires to grade-up New Zealand Ayrshires, which probably differ little from their Scottish counterparts. Continued adherence to this grading-up policy on a national scale implies either that stock deteriorate in the Dominion—and there is no evidence to support this—or that Scottish breeders are continually making improvements and preserving a favourable genetic margin between the representatives of the Ayrshire breed in the two countries.

ACKNOWLEDGEMENT

The above investigation was carried out with the assistance of a University Research Grant for which grateful acknowledgement is made.

Particulars of the animals imported into New Zealand from 1910 to 1949 have been deposited with the Secretary of the New Zealand Ayrshire Cattle Breeders Society.

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THE USE OF CHEMICAL THINNING SPRAYS ON APPLE TREES IN NEW ZEALAND

I. PRELIMINARY EXPERIMENTS IN HAWKE'S BAY AND NELSON

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(Received for publication, 2 November 1954)

Summary

Sprays of sodium dinitro-ortho-cresylate (DNOC), alphanaphthalene acetic acid (ANA) and sodium 2-methyl-4-chloro-phenoxyacetic acid (MCPA) were tested at a range of concentrations as chemical thinning agents on three apple varieties, Dougherty, Jonathan, and Sturmer. The experiments were conducted over two seasons, 1950–51 and 1951–52, in two principal New Zealand fruit-growing areas, Hawke's Bay and Nelson.

Both DNOC and ANA proved efficient thinning agents, but MCPA was unsatisfactory. In Hawke's Bay, suitable concentrations of both DNOC and ANA on Sturmer in 1951–52 gave a satisfactory commercial result, but in Nelson most treatments tended to overthin.

Jonathan seemed slightly easier to thin than Sturmer, whereas Dougherty was much more difficult.

Effective treatments in general notably increased fruit size on all varieties.

All chemicals tested gave some foliage injury, the severity of which depended on concentration, time of application, variety, and tree vigour.

In Hawke's Bay, early thinning by DNOC and ANA on Sturmer and Dougherty induced a heavier blossoming in the following year on sprayed trees than on check trees.

INTRODUCTION

Removal by hand of excess fruit early in the season to ensure well sized, evenly distributed fruits at harvest is one cultural operation in orchards that can be both laborious and expensive. A means of reducing hand thinning costs would be of great value to orchardists, and chemical thinning methods offer a solution to this problem.

Experiments in the United States and other countries have established the value of chemical sprays applied at blossoming or shortly afterwards for thinning apple crops. Much of this early work in the United States has been summarized by Batjer and Hoffman (1951). Advantages in obtaining such an early thinning of fruit are marked. Not only is hand thinning considerably reduced, but also fruit harvested is of larger size. Early reduction in crop has also been reported to reduce biennial crop fluctuations of certain apple varieties.

Two types of chemicals have been used widely for this purpose, namely dinitro compounds such as sodium dinitro-ortho-cresylate (DNOC), and synthetic growth substances such as alpha-naphthalene acetic acid (ANA). Dinitro compounds thin mainly by a caustic pollenicidal action on open blossoms and are thus restricted to use at full bloom. The thinning action of ANA, which is effective for a period from blossoming to several weeks later, is suggested by Luckwill (1953)

to involve at least two phases. The chemical may interrupt fertilization processes, thus preventing fruit set, or may cause seed abortion and drop of young fruitlets.

In the 1950–51 season, field experiments using chemical thinning sprays were commenced in Nelson and Hawke's Bay. These districts differ markedly in the amount of hand thinning required. In Hawke's Bay a heavy set is usual and most varieties are thinned to at least one fruit per cluster in an "on" year. By contrast, in Nelson only a light thinning is required on most blocks, exceptions being certain varieties such as Dougherty or Statesman.

Results of these investigations, which have been continued each season since 1950, are reported in this and subsequent papers of this series. Preliminary reports on some of this work have already been published (Davison, 1952, 1954: Davison and Robbins, 1953).

METHODS AND RESULTS

In all experiments, mature trees were used, each treatment being applied to 5 or 6 trees randomized within blocks as single-tree plots. For most trials, the method of recording the effectiveness of thinning treatments was to count and mark off at least 100 flowering clusters (points) on comparable branches before spraying, and to count the number of fruit remaining before hand thinning. At least two such marked limbs were selected on each tree. In some experiments the number of clusters bearing fruit and numbers of fruits occuring singly or in bunches of two were counted on these same branches. In other experiments the amount of thinning as judged by commercial standards was also scored by observation. Crop weights, together with the number of fruit harvested were recorded in the majority of experiments and an average fruit weight calculated. All sprays were applied with small portable spray units, using a double-nozzle lance and a pressure of approximately 400 lb./sq. in.

1950–51 SEASON

Nelson

Table 1 records the range of treatments and results obtained on two varieties, Jonathan and Sturmer.

Figures from the DNOC series on Sturmer are anomalous in that the 1½ pints/100 gal. concentration gave a greater reduction in fruit set than did the 2 pint/100 gal. concentration. Most effective treatments on the Sturmer were the post-blossom ANA sprays which caused marked thinning when applied even as late as three weeks after full bloom. The Jonathan variety seemed more easily thinned than Sturmer by both chemicals.

Reduction in the number of fruit per cluster, a measure of the ability of the sprays to thin bunches, was especially marked with post-blossom ANA sprays on both varieties. There seemed no consistent differences between ANA and DNOC sprays in their effect of completely eliminating fruit from clusters.

Fruit set was not heavy, consequently effective treatments resulted in overthinning and reduced yields.

TABLE 1. EFFECT OF THINNING SPRAYS ON STURMER AND JONATHAN APPLES IN NELSON 1950–51

		Sturmer (5 trees per treatment)					Jonathan (5 trees per treatment)				
		Av. Yield per Tree (lb.)	Av. Fruit Wt. (lb.)	No. Fruits per 100 Pts.	% Fruits in Singles and Doubles	% Clusters with Fruit	Av. Yield per Tree (lb.)	Av. Fruit Wt. (lb.)	No. Fruits per 100 Pts.	% Fruits in Singles and Doubles	% Clusters with Fruit
Unsprayed control		368.6	0.264	150.2	53.9	73.3	367.6	0.257	96.7	84.1	65.4
DNOC 1 pint/100 gal.	Full bloom 14/10/50	388.6	0.309	149.1	60.8	78.6	403.3	0.282	75.3	89.1	52.7
„ 1½ „ „		338.0	0.303	117.6	62.1	64.3	—	—	—	—	—
„ 2 „ „		385.2	0.324	135.1	59.3	69.3	279.6	0.327	65.3	88.9	46.3
ANA 5 p.p.m.		358.4	0.288	124.1	57.9	63.4	306.6	0.291	72.4	87.1	49.4
„ 10 „		315.8	0.336	109.6	55.8	55.3	—	—	—	—	—
„ 5 „	100% petal fall 26/10/50	—	—	—	—	—	295.2	0.293	61.7	95.3	49.8
„ 10 „		304.8	0.359	100.3	73.7	62.8	260.2	0.302	60.2	92.5	48.2
„ 20 „		232.7	0.379	79.2	77.1	51.2	—	—	—	—	—
„ 20 „ —8 days after petal fall 3/11/50		236.6	0.327	84.7	77.3	52.9	—	—	—	—	—
Diff. for sig. 5%		88.8	0.033	28.2	10.7	10.4	66.4	0.019	17.4	8.4	9.2
1%		119.2	0.044	37.8	14.4	14.0	89.9	0.026	23.6	11.4	12.5

1. DNOC sprays were prepared from Elgetol (containing 30% sodium dinitro-ortho-cresylate). An alcoholic solution of the pure chemical was diluted for the ANA sprays and a spreader Agral L was later added to the spray tank.
2. Full-bloom applications were made when (a) 80–90% of flowers on the Sturmers were fully open. (b) 90% of flowers on the Jonathan were fully open.
3. Weather for 4 days preceding application of full-bloom sprays was warm and sunny with little wind.

Treatments which thinned, in general, resulted in a significant increase in average fruit weight. Neither ANA or DNOC has given consistently larger fruit than the other. A point to be noted is the significantly smaller size of fruit from Sturmer trees sprayed with ANA 20 p.p.m. three weeks after full bloom compared with fruit from trees receiving a similar concentration of spray eight days previously.

No effect on fruit quality at harvesting was observed.

Hawke's Bay

Wet weather over the whole of the blossoming period prevented use of DNOC in Hawke's Bay during the 1950–51 season and only ANA sprays were applied.

Details of treatments and results are given in Table 2. Considerable thinning occurred on the Sturmers with both sprays, and a significant thinning of clusters to one or two fruits was achieved. Doughertys, however, proved more difficult to thin.

TABLE 2. EFFECT OF THINNING SPRAYS ON STURMER AND DOUGHERTY APPLES IN HAWKE'S BAY 1950–51

	(6 trees per treatment)		(5 trees per treatment)		
	Fruit per 100 Pts.	% Fruit in Singles and Doubles	Fruit per 100 Pts.	% Fruit in Singles and Doubles	% Clusters with Fruit
Unsprayed control	142.3	69.2	92.9	86.6	58.2
ANA 10 p.p.m.—100% petal fall 27/10/50	81.6	81.3	97.9	83.8	66.6
„ 20 „ —12 days later 8/11/50	94.6	80.3	70.5	93.6	53.4
Diff. for sig. 5%	23.0	9.7	34.9	10.7	12.8
1%	32.7	13.9	50.8	15.6	18.9

1. The ANA sprays were made up as shown in Table 1.
2. Weather over the whole of the blossom period was showery with moderate temperatures.

An estimate of the number of fruits setting per 100 clusters for a satisfactory crop was 55–60 on each variety, so that the degree of thinning achieved was inadequate. Subsequently all trees of both blocks required hand thinning.

The sprays appeared to have no effect on fruit quality.

Other Effects of Sprays

The DNOC sprays on Sturmer and Jonathan at Nelson caused marginal leaf scorch and browned withered petals, effects that were only

slight at the lower concentrations of 1–1½ pints per 100 gal. but were classed as slight to moderate with the 2 pints per 100 gal. application. Only early leaves were affected, and new growth rapidly obscured the damage.

All ANA sprays induced slight leaf curling and drooping of foliage on both Sturmer and Jonathan, but with the 5 p.p.m. concentration trees recovered within two weeks. With the 10 and 20 p.p.m. sprays, however, leaf curling was more marked and persistent. Sturmers seemed more susceptible to this effect and it was 8 weeks before foliage recovered as against 4 weeks with Jonathan. The effect was less marked when the ANA treatment was applied 3 weeks after full bloom.

Similar general effects on foliage following ANA sprays occurred on treated trees in Hawke's Bay although the injury seemed more persistent, being apparent throughout the season on Sturmer. Dougherty was not as susceptible as Sturmer and recovered within 8 to 10 weeks. On both blocks the more vigorous trees were less affected than weaker trees.

1951–52 SEASON

Nelson

A further trial was carried out on Sturmer during the 1951–52 season, details of which are set out in Table 3. In addition to DNOC

TABLE 3. EFFECT OF THINNING SPRAYS ON STURMER APPLES
NELSON 1951–52
(6 trees per treatment)

		Av. Yield per Tree		Av. Fruit Wt. (lb.)	Degree of Thinning
		lb.	log.		
Unsprayed control		300.7	2.4648	0.277	—
DNOC 1½ Pts/100 gal.—	Early full bloom 5/10/51	266.2	2.4158	0.330	Very slight thinning
„ 1½ „	Full bloom 8/10/51	249.1	2.3880	0.345	Moderate thinning
„ 2 „		207.4	2.3345	0.387	Gross thinning
ANA 5 p.p.m.		269.1	2.4082	0.303	Very slight thinning
ANA 10 p.p.m.	After petal fall 25/10/51	226.6	2.3534	0.314	Slight thinning
MCPA 10 p.p.m.		308.3	2.4829	0.258	No effect
Diff for sig. 5%			0.3747	0.027	
1%			0.5046	0.036	

1. The DNOC sprays were prepared from Selectone (containing 30% sodium dinitro-ortho-cresylate). Planofix and Agroxone, two commercial products based on alpha-naphthalene acetic acid and sodium 2-methyl-4-chloro-phenoxy-acetate respectively, were used as sources for these chemicals.
2. The early application of DNOC was made when approximately 60% of flowers were fully open, the later when 80% of flowers were fully open.
3. Fine warm conditions prevailed up to the first DNOC application. The following day a storm occurred with corresponding low temperatures but the remainder of the blossom period was fine and sunny.

and ANA treatments, the growth substance sodium 2-methyl-4'-chlorophenoxyacetate (MCPA) was tested.

Results given in Table 3 indicate the effectiveness of DNOC and ANA in reducing fruit set and increasing fruit size. The early DNOC spray was less effective than the later applications, the strongest of which grossly overthinned. MCPA 10 p.p.m. at petal fall was ineffective.

Both the 1½ pints per 100 gal. DNOC sprays gave uneven distribution of fruit compared with the ANA 10 p.p.m. which thinned out most bunches to one or two fruits.

Where necesary, some hand thinning was done on all treated trees to break bunches but the set on the unsprayed trees was not heavy and most treatments overthinned, especially at the bases of the trees.

Fruit quality at harvest did not seem to be affected by any of the sprays.

Hawke's Bay

Sturmer. A further trial including DNOC and ANA treatments was carried out on Sturmer in Hawke's Bay during the 1951–52 season. The concentration of ANA applied at petal fall was increased to 20 p.p.m. because of the unsatisfactory results of the 10 p.p.m. spray in 1950–51. Details of the treatments are given in Table 4 together with results.

A heavy set of fruit occurred on unsprayed check trees. Observations and detailed fruit-set counts indicated that all treatments excepting the 10 p.p.m. ANA full-bloom spray resulted in significant thinning. A good commercial crop for these trees would be approximately 55 fruits per 100 clusters, so that both DNOC sprays and the ANA 20 p.p.m. spray at petal fall reduced set to a satisfactory level. The 10 p.p.m. ANA full-bloom spray, however, underthinned while the 20 p.p.m. application at the same time caused gross overthinning.

All trees were hand thinned early in December; check trees required a heavy thinning whereas only odd bunches needed breaking on trees thinned down to a satisfactory level by the chemical treatments. The time necessary for this task on the latter trees was estimated to be only a third of that required on unsprayed trees.

Yields from all chemical treatments excepting the ANA 20 p.p.m. at full bloom were approximately similar to that of unsprayed trees, but overall fruit size was uniformly larger and there was little small fruit present. Because of drastic overthinning resulting from the ANA 20 p.p.m. full bloom spray, all fruit from these trees was outsize and commercially useless. The number of fruits per case is included in Table 4 as a more conventional way of expressing average fruit size.

At harvest, ground colour differences suggested that fruit from chemically thinned trees was ripening about two weeks before that from unsprayed trees.

Observations of the amount of blossom formed on trees of this block the following spring (1952–53 season) showed that all but the least vigorous of the sprayed trees bore a moderate blossom compared with a light blossom on unsprayed check trees.

TABLE 4. EFFECT OF THINNING SPRAYS ON STURMER AND DOUGHERTY APPLES IN HAWKE'S BAY, 1951–52

Sturmer (6 trees per treatment)		Av. Yield per Tree		Av. Fruit Wt. (lb.)	No. Fruit per 100 Pts.	Av. No. Fruit per Case
		lb.	log.			
Unsprayed control		357.3	2.5433	0.304	90.2	135
DNOC 1½ pts/100 gal.	Full bloom 11/10/51	350.2	2.5217	0.357	55.7	114
„ 2 „ „		332.7	2.5089	0.354	50.5	114
ANA 10 p.p.m.		354.2	2.5365	0.369	76.0	111
„ 20 p.p.m.		148.1	2.1545	0.532	17.9	79
„ 20 p.p.m.—100% petal fall 25/10/51		359.2	2.5330	0.328	60.8	123
Diff. for sig. 5%			0.1182	0.065	17.2	
1%			0.1601	0.088	23.4	
	ANA 20 p.p.m. Full bloom			0.070		
				0.094		

Dougherty (6 trees per treatment)		Av. Yield per Tree		Av. Fruit Wt. (lb.)	No. Fruit per 100 Pts.
		lb.	log.		
Unsprayed control		499.9	2.6727	0.225	119.1
ANA 20 p.p.m.	100% petal fall 25–27/10/51	407.4	2.6019	0.269	106.4
„ 30 p.p.m.		423.2	2.6236	0.275	84.1
MCPA 10 p.p.m.		422.2	2.6221	0.232	130.0
„ 20 p.p.m.		432.3	2.6244	0.239	118.3
„ 30 p.p.m.		338.2	2.5132	0.239	97.9
Diff. for sig. 5%			0.0354	0.017	27.0
1%			0.0479	0.023	36.0

1. All sprays were prepared as in Table 3.
2. The DNOC sprays were applied when approximately 80–85% of flowers were fully open, 10% were unopened and 5% were at petal fall.
3. Because of windy conditions which developed during spraying on 25/10/51, application of MCPA 20 p.p.m. and 30 p.p.m. on Dougherty was delayed until 27/10/51. Weather otherwise was cloudy and warm with occasional showers over the blossom period.

Dougherty. Various concentrations of ANA and MCPA were applied to Dougherty in Hawke's Bay in the 1951–52 season. Details of treatments and results are presented in Table 4.

In spite of a very heavy blossoming, fruit set on the Dougherty block was only moderate. None of the sprays achieved any observable thinning, although counts of fruit set indicated that the higher concentration of ANA resulted in a significant reduction. A moderate hand thinning was required on unsprayed check trees and all chemical treatments were supplemented by hand thinning.

The slight thinning on the ANA-treated trees did, however, give a significant increase in fruit size.

All sprayed trees showed a heavier blossom the following season (1952–53) than the unsprayed trees.

Other Effects of Sprays

Effects of sprays on foliage during the 1951–52 season were in general similar to those observed the previous year. Sturmer trees sprayed at early full bloom with DNOC were less affected than those treated three days later. Many undamaged blossoms later opened on these earlier-sprayed trees.

The MCPA sprays caused effects similar to those of ANA and also induced some malformation of developing spur and shoot leaves. Subsequent growth, however, was normal. This treatment also caused the retention of numerous small fruitlets on the spurs for some two months after spraying.

A feature of the Hawke's Bay experiments was the severe permanent curling and inrolling of Sturmer leaves following application of ANA 20 p.p.m. at full bloom. This treatment also caused a marked reduction in size of the first leaves. The effect of other ANA sprays was slight and became insignificant within a few weeks, in contrast to the persistent curling noted during the 1950–51 season in Hawke's Bay.

DISCUSSION

These results demonstrate that both DNOC and ANA will thin apple trees under New Zealand orchard conditions. DNOC sprays were more effective when applied at late full bloom, i.e. when 80 to 90% of blossoms were at full bloom, than just prior to full bloom when only 60% were at full bloom, indicating the necessity for careful timing of applications. In practice, a difficulty could arise in determining exactly the most suitable application time, since blossoming in most New Zealand areas occurs over an extended period of up to ten days. The short period over which DNOC must be applied for optimum effectiveness is also a serious disadvantage under the weather conditions normal for both districts at that time of year. This is emphasized by conditions over the blossoming period in Hawke's Bay during the 1950–51 season when DNOC applications were prevented by bad weather.

In contrast, ANA sprays have a period of effectiveness ranging from full bloom to at least three weeks later, although they achieve a much greater effect at full bloom than at petal fall or later. Thus timing of ANA sprays is not as critical as for the dinitro compounds, and any delay in application can be overcome by an increase in spray concentration. Moreover, later application enables a better estimate of fruit set to be made, with a consequent better assessment of the amount of thinning necessary.

MCPA did not thin satisfactorily, although it has been reported to give good results in Australia (Bowman *et al.*, 1948).

It is evident that chemical thinning sprays will thin out individual bunches of fruit as well as eliminate fruit from entire clusters (Table 1), and under conditions where heavy hand-thinning is necessary the result

can be a satisfactory distribution of fruit over the tree. No consistent difference between ANA and DNOC sprays was shown in this respect.

Timing of the ANA application appears to influence the degree to which bunches may be thinned, for this effect was greater with post-blossom sprays than with full-bloom sprays. Brough (1951) notes a similar result on the variety Yates in Victoria, Australia.

A tendency for the sprays to remove fruit more easily from the base of trees was noted, particularly when overthinning occurred. This effect, which is of some significance in commercial applications, could be largely overcome by varying the quantity of spray on different parts of the tree.

A differential varietal response to thinning sprays is apparent from these experiments, emphasizing that no one concentration of spray is likely to be suitable for all varieties in an orchard. In Nelson, Jonathan appeared to be slightly more easily thinned with both chemicals than Sturmer, while in Hawke's Bay Dougherty was much more resistant to ANA than Sturmer.

A comparison of results obtained on Sturmer in Hawke's Bay and Nelson indicates a great difference in the concentration of sprays, especially ANA, required for a satisfactory commercial thinning. For example, in the 1951–52 trials, a concentration of 20 p.p.m. ANA was necessary in Hawke's Bay whereas a concentration of 10 p.p.m. in Nelson slightly overthinned. Such a contrast emphasizes that thinning sprays are likely to be of much greater benefit in Hawke's Bay than in Nelson.

No indication was obtained of any relation between seasonal conditions and effectiveness of sprays. Both 1950–51 and 1951–52 seasons were, however, considered normal seasons and such an effect might therefore not have been expected. This seasonal factor is considered of some importance by Batjer and Hoffman (1951). The evidence they have summarized suggests that a much greater thinning effect can be expected from both growth-substance and dinitro sprays in cool humid weather or when trees are affected indirectly by seasonal conditions that prevent a good set of fully seeded fruits.

A certain degree of foliage injury from thinning spray seems unavoidable. Both types of chemical cause damage that varies with concentration, time of application, variety, and tree vigour. Thus Dougherty appears much more resistant to ANA injury than Sturmer. The degree and persistence of foliage effects caused by ANA may also vary between seasons. Although no differences were observed in these experiments, DNOC injury is also reported to vary greatly, being more severe in a cool wet spring (Batjer and Hoffman, 1951).

Foliage damage on Sturmer and Dougherty did not prevent early thinning from promoting an adequate number of flower buds for a full crop the following season. Even the severe dwarfing of foliage following treatment with ANA 20 p.p.m. at full bloom did not reduce blossom formation compared with other spray treatments, although this result is contrary to American experience. These results indicate that early spray thinning may reduce the biennial cropping tendency of Sturmer and Dougherty in Hawke's Bay.

Average fruit weights were in general increased by effective treatments and this result demonstrates one of the chief advantages of chemical thinning, i.e. the achievement of a larger fruit size. In Hawke's Bay experiments, treated Sturmer trees yielded fruits that on the average were at least one packing size larger than those from unsprayed trees (Table 4). Size increases of this order are likely to be of great value with a variety such as Dougherty where fruit sizing is often a problem. Although chemical thinning may not remove as much fruit as desired, e.g. on the Dougherty block in 1951–52, the early elimination of a proportion may still bring about a significant increase in fruit size.

Although it might be expected that DNOC full-bloom sprays would give a greater increase in fruit size than post-blossom ANA sprays, no consistent difference between ANA and DNOC was shown in this respect. Luckwill (1951) demonstrated that under certain conditions late post-blossom ANA sprays directly inhibited fruit growth. There was no clear indication of this effect in these trials, although there seems some evidence for it in a comparison of the two 20 p.p.m. ANA sprays applied to Sturmers (Table 1).

The apparent effect of chemical thinning sprays on fruit maturity is of some importance, as Sturmer is one of the main storage varieties grown in New Zealand. Such an effect on maturity has been observed in the United States (M. B. Hoffman, A. E. Murneek, pers. comm.) but mainly on early varieties, which are not stored for long periods.

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NUMBERS AND WEIGHTS OF EARTHWORMS UNDER A HIGHLY PRODUCTIVE PASTURE

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Summary

1. Seasonal variations, over a period of 2½ years, in the total weight of earthworms per square foot, and in the weights, numbers, and average weights of *Allolobophora caliginosa* and *Lumbricus rubellus* in a highly productive pasture at Grasslands Division, Palmerston North, are presented graphically and described. Separate data are presented for earthworms under and over 3 cm. in length.
2. Soil moistures, 4-in. ground temperatures, and dry-herbage production figures for the 2½-year period are presented graphically.
3. Pot experiments are described showing the negligible effect upon the above species of earthworms of the seasonal variations in temperature occurring at Palmerston North, and evidence is drawn from the graphs in support of the conclusion that soil conditions associated with high soil moisture probably did have an adverse effect upon the earthworms.
4. It is concluded that the seasonal rises in the earthworm weights and numbers were chiefly caused by seasonal flushes of dead root and to a lesser extent dead herbage debris, the seasonal decreases in the earthworm weights and numbers being attributed to the natural exhaustion of these flushes and to the adverse effect upon the earthworms of the conditions of poor aeration that accompany high soil moisture.

INTRODUCTION

There is accumulating evidence that earthworms stimulate the growth of pasture plants. The weights and numbers of earthworms in pasture is therefore of increasing interest.

Evans and Guild (1947) studied the seasonal changes in the number of earthworms in a grass pasture on a silty medium loam at Rothamsted. The earthworms were expelled from the soil with a solution of potassium permanganate. The counts show great variability between successive samplings which the authors suggest was due primarily to limitations of the potassium permanganate method. They found the earthworm population to be highest in the autumn and lowest in the summer, and higher in spring than in winter.

They also studied the seasonal activity of earthworms by collecting worm casts at about four-day intervals. The data obtained in this way are unlikely to have given an adequate picture of earthworm activity as a whole, because, of the six species present, only *Allolobophora nocturna* and *Allolobophora longa* cast on the surface.

Hopp (1947) sampled hay pasture and tilled corn plots on a Sassafras loam in Maryland by removing cores, approximately 7 in. in diameter and 7 in. deep, at approximately monthly intervals for a year. This method is open to the objection that it is not known what proportion of the earthworm population present was below 7 in., nor whether this proportion varied throughout the year. Under sod, the greatest weight of earthworms occurred in the spring, the greatest number in early summer, and the least weight and number in the autumn.

It is impossible to decide whether the differences between the English and American observations are due to climatic factors, to the type and productivity of the pasture studied, or to differences in technique. Moreover, seasonal trends are difficult to define in trials lasting a maximum period of 16 months.

It was found (Waters, 1951; Sears and Evans, 1953) from samples taken in spring at Palmerston North and in winter at Gore that the weight of earthworms under a pasture was almost directly proportional to the annual dry-matter production of the pasture. Herbage production in the plots ranged from 1,000 to 15,000 lb. of dry matter per acre per annum. The soil contained approximately 170 lb. of earthworms per acre per 1,000 lb. of annual dry-matter production, and thus up to 2,400 lb. of earthworms per acre. It was not known, however, whether the weights recorded were annual maxima or not, nor how the population varied throughout the year under New Zealand conditions. Accordingly, a study of the earthworm population in a highly productive pasture was undertaken to determine the extent and pattern of seasonal variations.

Experimental Method

This study began in June 1951 and continued for a period of 2½ years. The area under observation consisted of 1/20 acre of 8-year-old pasture of perennial ryegrass and white clover grazed by sheep at Grasslands Division, Palmerston North. The dry-herbage production per acre of this area was 13,900 lb. in 1951 and 15,180 lb. in 1952. The soil is classified as Manawatu silt loam of recent origin (Taylor N. H. *et al.*, 1954).

The area was sampled once a month by cutting out 33 randomly distributed plugs 6.9 in. square (i.e. ⅓ sq. ft.) and 1 ft. deep. This number of samples was adopted to reduce the standard error of the mean weight of earthworms per square foot to less than 5%. Each plug was divided into three 4-in. layers. If any earthworms were discovered in the bottom layer further layers were removed until it was clear that all the earthworms in the sampled area had been recovered. Since the majority of earthworms occurred in the top or middle 4 in., a 12-in. depth, or at most a 16-in. depth, was sufficient. Sampling was at approximately monthly intervals, and took 3 days, 11 plugs being dug up and dissected each day. The earthworms removed were stored in moist glass jars until the fourth day. They were then separated into their two constituent species, *Allolobophora caliginosa* and *Lumbricus rubellus* and grouped into small worms (up to 3 cm. long) and large worms (3 cm. and over). The worms in each category were then counted, after which the small and large worms of each species were bulked and weighed to give the total weight of each species. The weighing was done on the fifth day, the guts of the earthworms being by then almost empty. While in the scouring jars the earthworms were washed daily to prevent self poisoning and were stored in the dark. In summer the jars were stored in a cool room.

Soil moistures were determined each week from 35 soil plugs each 1 in. in diameter and 3 in. deep, dried in an oven at 110°F. Mean monthly ground temperatures at 4 in. were calculated from the records of the local meteorological station situated about 400 yd. from the experimental area, it having been found that the soil temperatures at

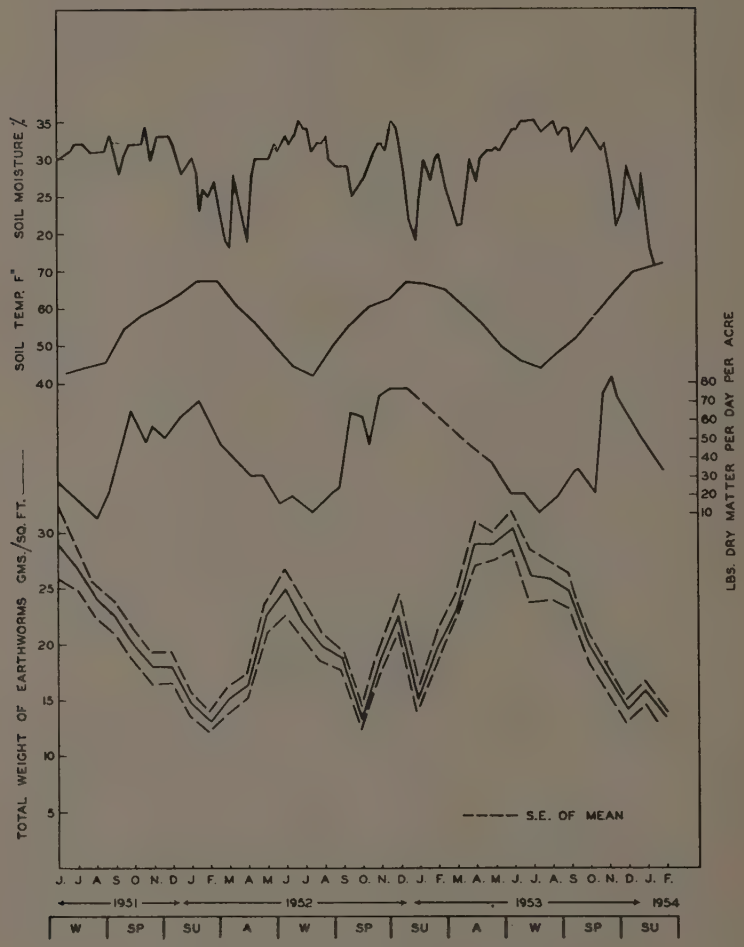
SOIL MOISTURE %
SOIL TEMP. F°
TOTAL WEIGHT OF EARTHWORMS GMS./SQ. FT.
LBS. DRY MATTER PER DAY PER ACRE
S.E. OF MEAN
J. J A S O N. D J F. M A M J J A S O. N. D. J. F. M A. M. J. J. A. S O. N. D. J. F.
1951
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Fig. 1.—Seasonal variation in total weight of earthworms per sq. ft.

4 in. were the same at the station as on the area. The area was grazed at intervals of approximately 3 weeks. The dry-herbage production of the experimental area was determined from the herbage yields of two 10 ft. × 4ft. sample areas, protected from stock by netting-covered frames. These areas were harvested immediately after each grazing.

Standard errors were calculated on the records of total earthworm weights per square foot.

RESULTS

Total Weight of Earthworms

Changes in total weight of earthworms per sq. ft., pasture production, 4-in. soil temperatures, and soil moistures, are shown in Fig .1.

Total weight of earthworms fell to a low level of about 13 g. per sq. ft. in late summer and increased rapidly in the autumn to reach a maximum of 25 to 30 g. in the early winter. Thus the total weight doubled over a period of 4 to 5 months.

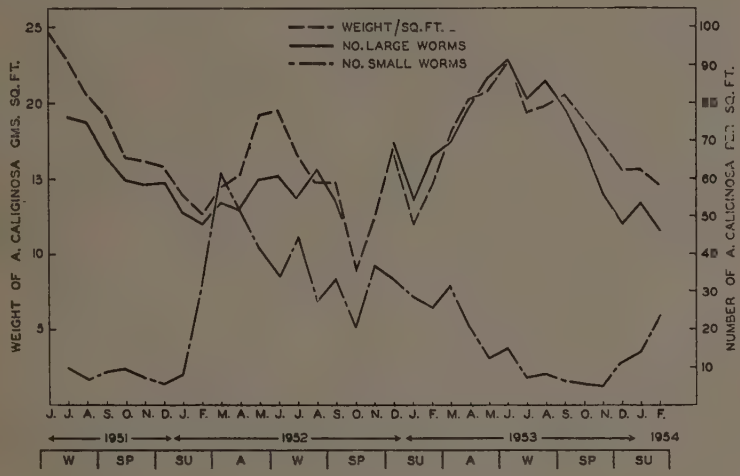
WEIGHT/SQ.FT.
NO. LARGE WORMS
NO. SMALL WORMS
WEIGHT OF A. CALIGINOSA GMS. SQ.FT.
NUMBER OF A. CALIGINOSA PER SQ.FT.
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Fig. 2.—Total weight of *A. caliginosa* per sq. ft.

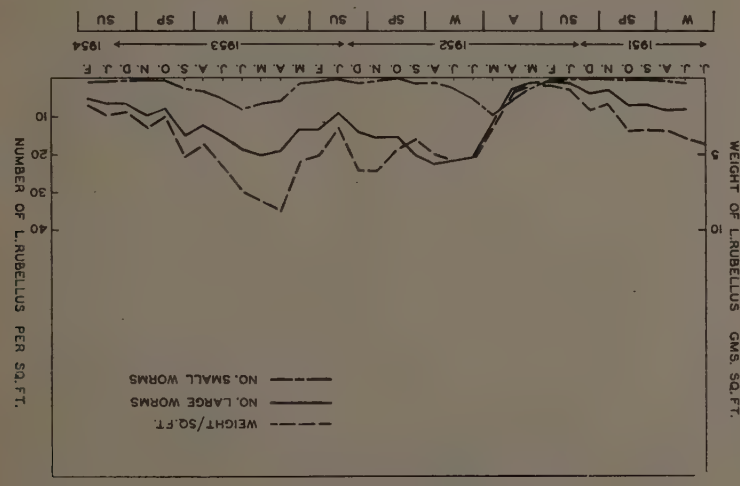

Fig. 3.—Total weight of *L. rubellus* per sq. ft.

Variations in Weight and Numbers of Different Species

There were generally only two species of earthworm in the pasture examined, *Allolobophora caliginosa* and *Lumbricus rubellus*. Very occasionally an individual of *Octoclasium cyaneum* was found. All these

worms are of European origin. *A. caliginosa* was the dominant species, constituting, over the whole period, 80.6% of the population by weight and 86.3% by number.

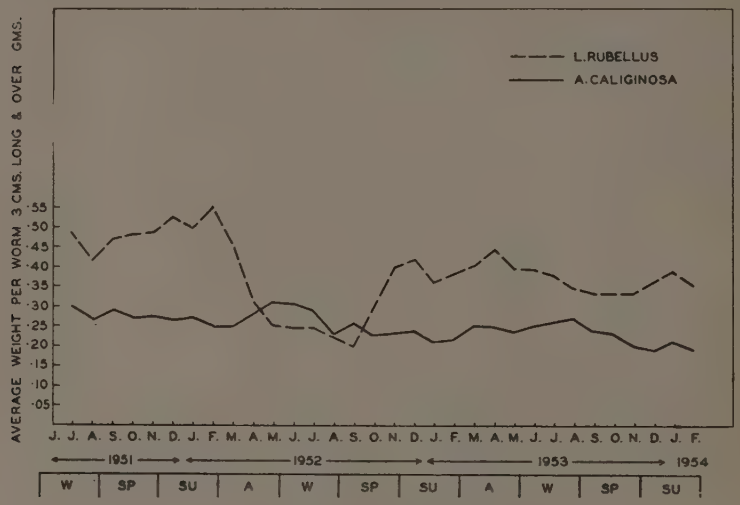

Fig. 4.—Average weight per worm.

Both species showed roughly the same seasonal trends in total weight (Figs. 2, 3) but the variations in total weight of *L. rubellus* (± 56% of the mean) were relatively greater than those of *A. caliginosa* (± 33% of the mean). This larger variation in total weight of *L. rubellus* is associated with larger variations in both numbers and average weight of worms (Figs. 3, 4).

Numbers of large *L. rubellus* also follow a fairly regular seasonal pattern (Fig. 3) but the numbers of large *A. caliginosa* (Fig. 2) have a less definite seasonal pattern owing to their having been limited in the autumn of 1952, presumably by the death of small worms in drought.

Numbers of small *L. rubellus* rose to a high level during the autumn (Fig. 3), presumably on account of high hatching rates at this time, and declined steeply during the winter. In the spring and summer small worms were scarce.

Numbers of small *A. caliginosa* (Fig. 2) showed no seasonal pattern nor could any relationship be discovered between the numbers of small worms and the numbers of large worms.

Both species showed a seasonal pattern of variation in the average weight of large worms (Fig. 4). The average weight of the large *A. caliginosa* rose in the autumn and winter though numbers also rose, and fell in the spring and summer though numbers fell. On the other hand, the average weight of the large *L. rubellus* fell in the autumn and winter, the influx of young worms being so much greater than in the case of *A. caliginosa*, and rose in the spring and summer when small worms were scarce. It is clear, therefore, that the pattern in the average weight of

L. rubellus was chiefly the result of the relatively greater effect on the *L. rubellus* weights of abundance and scarcity of small worms.

Pot Experiments

As part of a series of earthworm feeding trials, pot experiments with earthworms have been carried out at all seasons. These experiments were conducted in a roofed open-sided outhouse, soil temperatures in the pots being governed, therefore, by the prevailing air temperatures. Each of these experiments contained at least one treatment in which large amounts of dry, powdered sheep dung was incorporated in the soil before the earthworms (*A. caliginosa*) were introduced. The increases in earthworm weight in these treatments are shown in Table 1.

TABLE 1. INCREASE IN WEIGHT OF WORMS

Exp. No.	Experimental Period	Dry Weight Of Soil (g)	Dry Weight Of Dung (g)	Number of Earthworms	Initial Total Weight of Worms (g)	% Increase in Total Weight of Worms
1	25/2/49—25/4/49	7500	192	10	4.5	124
2	24/5/49—26/7/49	7000	200	25	7.6	121
3	10/8/49—11/10/49	6000	200	100	31.6	66
4	16/12/50—13/2/51	1500	71	50	17.3	60

DISCUSSION

Weights and numbers of earthworms found in this study are considerably higher than those quoted by Evans (1948) and Hopp (1947) for English and American pastures respectively. The former records numbers varying between 600,000 and 750,000 weighing from 800 to 1,000 lb. per acre; the latter states that figures up to 450,000 and 560 lb. were not uncommon. In the present investigation worm numbers ranged between 3 and 5 million per acre and worm weights between 1,300 and 2,700 lb. These high figures are undoubtedly due to the high productivity of the pasture, and the fact that the soil and climatic conditions that favour the growth of such highly productive pasture are just those that exclude extremes of soil moisture and soil temperature, which other workers have concluded to be adverse to earthworms.

The factors that must be considered as possible causes of the seasonal variations in weight and number of earthworms present are soil temperature, soil moisture, changes in food supply, and factors connected with the life cycle of the species concerned, such as periodicity of breeding and hatching, rate of growth, longevity, etc.

Little is known of the life cycle of *A. caliginosa* and *L. rubellus* under New Zealand conditions. There is, however, evidence in Fig. 3 of seasonal periodicity in the hatching rate of *L. rubellus*, but the seasonal influx of young worms in the autumn of each year is not of itself sufficient to explain the seasonal increases in the numbers of large worms, nor is the scarcity of young worms during the spring and summer

of each year sufficient to explain the seasonal decreases in the numbers of large worms, since only in the spring of 1952 when total weight increased and number of large worms remained constant was this demonstrably a limiting factor.

In Evans and Guild's study (1947), soil moisture varied from about 15% to 47% and soil temperature from 32° to 67°F., and they reported that there were clear responses to changes in soil temperature and soil moisture in all species. In Hopp's study (1947), the maximum air temperature was below freezing for two days. On tilled plots the soil froze to an average depth of 4 in. on 2 December, but on sod and mulched plots soil temperatures were above freezing. On tilled plots the earthworm population decreased by approximately 50% during the freeze while in the sod plots the earthworms increased by 23%. It was not until the latter half of January that the soil froze deeply under sod, and by that time the earthworms had become tolerant of freezing temperature. Hopp estimates the optimum temperature for earthworms to be about 36°F.

On the Manawatu silt loam at Grasslands, 4-in. soil temperatures ranged from 39° to 76°F., summer temperature varying between 60° and 70°F. and exceeding 70°F. for more than three consecutive days only in the summer of 1954. Winter temperatures should not have been detrimental to the earthworms, and, since in the feeding trials increases in earthworm weight of from 60% to 120% were recorded at all seasons, it is improbable that summer temperatures were detrimental either. Fig. 1 shows that earthworm weights tended to fall during periods of rising soil temperatures and to rise during periods of falling soil temperatures, but, in view of what has been said above and in view of the fact that in the spring of 1952 earthworm weights increased by 72% while temperatures were still rising, it seems improbable that this relationship was a causative one.

There is also evidence from pot experiments conducted at Grasslands that high soil moistures are not detrimental to earthworms provided there is free drainage of excess water. In pasture, however, high soil moisture is often associated with adverse conditions of low soil aeration set up by burrow flooding and surface pugging by stock. These conditions occurred in the pasture sampled, and may well have had an adverse effect upon the earthworm population. This is the more likely since periods of high soil moisture were usually followed by a decrease in earthworm weights (Fig. 1). It is possible too that low soil moisture, by causing the *A. caliginosa* to stop feeding and go into diapause, as they regularly did each summer, also had adverse effects upon the earthworm weights, but, droughts being of short duration and never very intense, these effects do not show up clearly in the data. Thus, while it is apparent that conditions associated with high soil moisture were detrimental to earthworms, there is no evidence that low soil moistures were detrimental.

Earthworm weights did not, however, fall immediately when soil moisture became high, but approximately a month later, nor did they rise immediately when soil moisture became moderate again, but continued to fall for one to two months. It is clear, therefore, that though the earthworms may have been adversely affected by high soil moisture

they were not controlled by this closely. It is also clear that moderate soil moisture is not all that is required to produce a rise in earthworm weights.

The size of a population may be limited by unfavourable environmental conditions or by food supply. The fact that this study does not indicate close control of seasonal trends in the total weight of earthworms, either by environmental conditions of by a marked annual hatching cycle, suggests that a varying food supply may have been the main controlling factor.

It was shown in a previous paper (Waters, 1951) that the weight of earthworms that a pasture will support is almost directly proportional to its dry-matter production, and it was concluded that this was so because the more productive a pasture is the more food it can provide for earthworms. But pot experiments have shown (R. A. S. Waters, unpublished data) that earthworms do not eat live herbage or live roots, since they lost weight in pots where these were the only organic materials supplied, and since no plant damage was observed under these conditions. The earthworm food that a pasture provides must consist, therefore, of the dead pasture debris. Consequently, earthworm weights are related to the production of dry herbage, not directly, but through the production of dead pasture debris.

This pasture debris consists of dung, dried herbage, and dead root. About a quarter of a pound of dung is returned by the grazing animal for each pound of dry herbage eaten. The amount of herbage debris produced depends upon weather conditions and grazing management. The amount of root debris is proportional to the amount of root growth, which is generally one third to one half as great as the herbage growth. The rate at which roots replace themselves is not, however, known. In any case the amount of root debris available may support a greater weight of earthworms than would at first be expected since the maintenance requirement of a cold-blooded animal is probably less than that of a warm-blooded one, and since the fact that an earthworm can extract nourishment from another animal's droppings testifies to its greater digestive efficiency. (Earthworms gained slightly more weight on dried sheep dung than on an equal weight of dried herbage, ground and mixed into the soil.)

Although not least in amount, dung would appear to be the least important of these food materials, previous studies having shown (Waters, 1951) that when the sheep dung produced from a pasture is returned to it, at least when it is returned in powdered form, it increases the weight of earthworms present by little more than 1 cwt. per acre, whereas we are looking for a source of food that can support 12 to 24 cwt. of earthworms per acre. It may be, however, that the above estimate of the importance of dung is on the conservative side, since Watkin (1954), in preliminary studies, has found that sheep dung returned to pasture in the normal way considerably increases the earthworm population. But if dung is a major source of earthworm food we would expect, since dry matter production and hence dung production is about 5 to 6 times greater in the spring and summer than in the winter, that earthworm weights would rise in the spring instead of falling and would reach their peak in the summer. And even if much of the dung dropped in the spring and summer did not become available to the earthworms until the autumn, there still remains the observation (Waters, 1951) that our pastures will

support about 170 lb. of earthworms per 1,000 lb. of dry matter even if no dung is returned at all. It is concluded, therefore, that dung was not a major source of food in this study, with the proviso that it may be a major source of food in other studies involving other earthworm species.

Herbage debris is probably not the chief food of earthworms either, since, although feeding experiments show that decaying herbage is consumed, the amount of herbage debris that remains after the stock have grazed a pasture is too small to support a weight of earthworms averaging 2,000 lb. per acre and equal in weight to the weight of the stock carried. It appears, therefore, that dead root debris must be the chief food of earthworms.

It has been found that new roots are produced most abundantly during the winter and spring, whereas during the late summer and autumn there is a considerable death of roots, which in a vigorously growing pasture tend to disappear from the soil by about the middle of the following winter (E. A. Jacques, pers. comm.). These findings are confirmed by Stuckey (1941), Troughton (1951), and others. It has also been found that there is a considerable death of tillers during and after dry periods, particularly if these occur in the summer and early autumn when the grasses are in their reproductive phase (K. J. Mitchell, pers. comm.).

Earthworm weights usually rise during the late summer, autumn, and early winter, when root and herbage debris is greatest in amount and dung and herbage production relatively low, and fall during the late winter, spring, and summer, when dung and herbage production is greatest in amount (Fig. 1). Thus the variations in earthworm weights are in closer accord with the variations in the supply of pasture-plant debris than they are with any other factor. It is probable, therefore, dead roots being the chief food of our earthworm species, that it is the increase in the supply of dead plant debris that accounts for the rises in the earthworm weights, and that it is the exhaustion of this supply that is mainly responsible for the falls in the earthworm weights.

The rise in earthworm weights that occurred in the spring of 1952 can be explained on this hypothesis since the high soil moistures of winter and spring were interrupted by a short dry period which, according to field observations at the time, induced considerable tiller death, with accompanying root death, earlier in the year than usual. The additional supply of food that thus became available, plus any surface accumulation of dung and herbage debris that may have been previously unavailable on account of weather conditions unfavourable to surface feeding, presumably induced the rise in earthworm weights that then took place.

A further illustration of how earthworm weights can be influenced by the supply of pasture-plant debris is probably to be found in the fact that the rises in the weights of *L. rubellus* are of greater relative magnitude than the rises in the weights of *A. caliginosa*. This is thought to be because, as feeding trials have shown, *L. rubellus* feeds more readily on herbage debris at the surface than *A. caliginosa*, and has, therefore, at those times when both root and herbage debris are available, a more plentiful food supply. On the other hand, when only dead root debris is available *L. rubellus* must be at a disadvantage, since this species does not thrive as well as *A. caliginosa* on subsurface feeding.

It is concluded, therefore, that under the climatic conditions and with the earthworm species found at Grasslands, the seasonal increases in earthworm weights and numbers are caused mainly by seasonal flushes of dead root and to a lesser extent dead herbage debris, while the seasonal decreases are due, either to the exhaustion of these food flushes, or to the adverse effect of the poor soil aeration accompanying high soil moistures, or to both of these factors in conjunction.

Large increases in weight unaccompanied by proportional increases in number of large worms show, however, that the number of earthworms present has occasionally been limited by other factors such as death of young, as in the case of *A. caliginosa,* or insufficiency of hatchings, as in the case of *L. rubellus,* but there is no evidence that these limitations were seasonally imposed.

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